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Cation Profiling in Normal Nasal Mucus

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Cation Profiling in Normal Human Nasal Mucus

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Declaration

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. All sources are acknowledged by bibliographic references. This work has not previously been accepted in substance for any degree and is not concurrently submitted in candidature for any degree unless, as agreed by the University, for approved dual awards.

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I would also like to thank my friends and family, especially my husband Dave, for always believing in me.

Abstract

Nasal mucus is common to all humans, yet little is understood about its composition. It is a special fluid produced by goblet cells. Analysing blood is quick, straightforward and relatively painless. Routine tests can be performed, and results obtained easily. There are no such tests for cation concentrations in nasal mucus.

Previous research has focussed on the 4 main bulk cations, Na^{+}, K^{+}, Mg^{2+} and Ca^{2+}, using varying techniques and obtaining a wide range of results. These studies used small numbers of participants in their studies. Only one of these studies has looked at trace metals within mucus.

This research has been carried out with funding from KESS2, the European Social Fund and company sponsor Halen Môn. The research has investigated a new method for mucus collection, using cotton wool pellets, and has been shown to be an effective efficient collection method. Samples were collected and analysed from 40 students, 20 male and 20 females, giving 80 samples. The analysis of these samples via inductively coupled plasma mass spectroscopy (ICP-MS) and atomic absorption spectroscopy (AAS) gave consistent results falling in line with previous results for bulk cations. Reproducible values for trace metals Cu^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, and Al^{3+} were obtained. These give a baseline composition for 'healthy' subjects. This was original research that had not been previously reported.

Natural nasal spray products, seawater and saline-based, are readily available to purchase from leading pharmacies and supermarket. Analysis of 10 of these products was also performed to look at their cation composition, as their contents are only alluded to. These values were used to see how the cation composition in nasal products compared to those in nasal mucus.

Abbreviations

AAS	Atomic Absorption Spectroscopy
AR	Allergic Rhinitis
CF	Cystic Fibrosis
cm	Centimetre
CW	Cotton wool
DDH_2O	Double Distilled De-ionised Water
HM	Halen Môn
IC	Ion Chromatography
ICP-MS	Inductively Coupled Plasma – Mass Spec
ICP-OES	Inductively Coupled Plasma – Optical Emission Spectroscopy
IgA	Immunoglobulin A
IgB	Immunoglobulin B
IgG	Immunoglobulin G
L	Litre
LOD	Limit of Detection
LOQ	Limit of Quantification
MRI	Magnetic Resonance Image
mEq/L	Milli equivalents of solute per litre of solution
mg/dL	milligrams per decilitre
mL	Millilitre

mm	Millimetre
mmol/L	Millimol per Litre
mOsmol/kg	Milliosmols per kilogram of water
ng/mL	Nano gram per mililitre
PBS	Phosphate Buffered Saline
pH	$-\log_{10}[H^+]$
RCT	Randomised Controlled Trial
SD	Standard Deviation
SEM	Standard Error of the Mean
µg/L	Microgram per Litre
µmmol/L	Micromol per Litre

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1 Introduction and Aims

To date there has been little research into the composition of nasal mucus. It is believed that the electrolyte balance, and the pH in nasal mucus, may play an important role in nasal health. It is thought that the balance of cations, particularly Mg^{2+}, may also contribute to conditions such as nasal polyps.[1] These common, noncancerous, teardrop-shaped growths form in the nose or sinuses, cause suffering to a large number of the population. Allergic rhinitis[2] symptoms may also be linked to an imbalance of cations within the nose.

Many, varied, attempts have been made to collect nasal mucus for analysis, some more successful than others. Methods for collection include nasal washing,[3] strips of filter paper inserted into the nasal cavity,[4,5] squares of gauze,[6] ion exchange beads on filter paper,[7,8] and cotton wool.[8] Secretions have been obtained in different ways; spontaneous secretions,[9] from a sneeze, at cold temperatures, and from subjects presenting cold symptoms.[10] Additionally, the subject group numbers in many cases are very low. In one study this was just the author,[10] two further had only 10 subjects,[4,5] while the largest study had 60 subjects.[6] The data collected these ways has been inconsistent, with values for electrolytes varying considerably from paper to paper. This will be discussed in greater detail in section 1.8.

The purpose of research presented within this thesis was to gain a greater understanding of the composition of nasal mucus in healthy human subjects, and to investigate a more efficient, less invasive, method for its collection. Better collection methods could enable a simple test to assist in a more effective treatment for patients. The intention had been to collect samples from patients with nasal polyps and allergic rhinitis. Ethics had been approved for this but was curtailed due to the onset of the COVID-19 pandemic. This knowledge could also facilitate the manufacture of a range of products to improve nasal wellbeing.

The project was conducted in conjunction with Halen Môn, a local sea salt producer. They were interested in diversifying their range of products to include a range of nasal wellbeing product featuring Halen Môn sea salt or seawater. Part of the project was to collect reliable data on the composition of competitor products on the market.

1.1 **The Nose**

The nose comes in different shapes and sizes. It contributes to hearing, tasting and plays an important part in the respiratory system. The nose is the passageway for air; oxygen is inhaled through it and carbon dioxide is expelled. Inhaled air is conditioned and warmed to make it more humid. It also acts as a filter to stop small particles entering the lungs.[11]

Figure 1[12] and Figure 2[13] display the basic anatomy of the nose as well as a more detailed cross section of the nose. The nose has two nostrils, separated by the septum, which is a wall of cartilage. It stretches to the back of the skull, above the oral cavity, inside the cheekbones and between the eyes with a framework of air cavities and canals, the sinus cavities. The nose in males is typically larger than those in females.

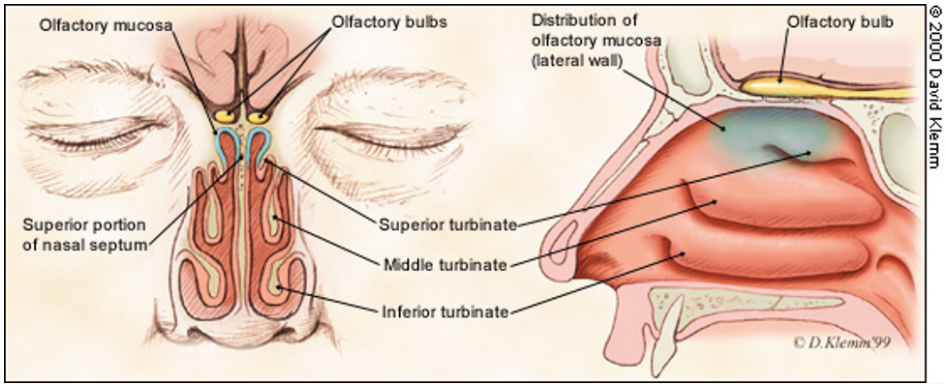


Figure 1 - Basic anatomy of the nose[12]

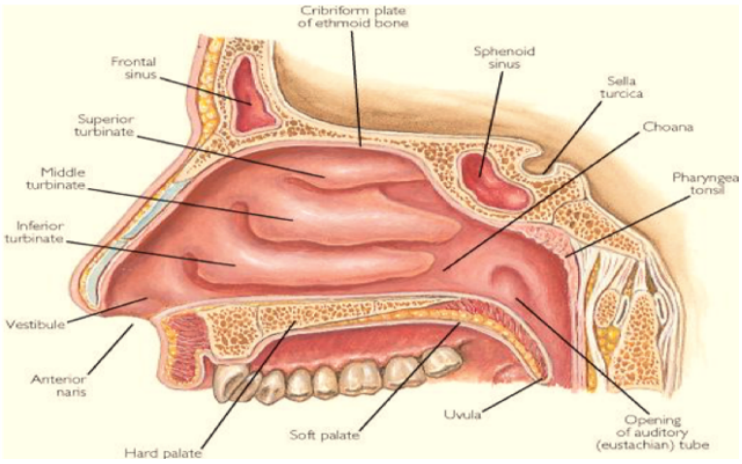


Figure 2 – Detailed cross section of the nose[13]

In healthy human subjects, nasal mucus is clear and very viscous. Mucus is secreted through the mucus membranes in the nose. Mucus can become thicker, even more viscous, and may change colour, while a person is experiencing the symptoms of a cold. A change in colour can be an indicator to the presence of a viral or bacterial infection.[14] At rest, 0.5 – 1.0 mL of mucus / cm^2 mucosa is produced in a 24h period. Mucus keeps membrane cells and the cilia, tiny hairs on the membrane surface, lubricated. The cilia help remove small particles such as dust, bacteria, allergens and viruses that have been inhaled and trapped in the layers of mucus that cover the cilia. The cilia transport the mucus to the throat where it is either swallowed or expelled as spit.[15]

Nasal Mucus is composed of water, glycoproteins, antibodies, enzymes and electrolytes (cations and anions) in 95% water.[16] These proteins include albumin,[17] lactoferrin[18] and mucin.[19] Immunoglobulins A, G and M are antibodies, with G being the most common form. Nasal mucus has an antibacterial property due to the presence of antimicrobial enzymes, lysozyme and lactoferrin.[20]

Bulk cations present include Na^+, K^+, Ca^{2+}, and Mg^{2+}, while anions include Cl^-, HCO_3^- and PO_4^{3-}.[10] The ions present play important roles in human physiological processes. An example is that K^+ and Na^+ regulate the water balance and the acid-base balance in the blood and tissues[21] [22]. Trace metal ions such as, Zn^{2+}, Fe^{2+}, Cu^{2+} and Mn^{2+} can also be seen in low concentrations.[23,24]

1.2 **Fluids in the Human Body**

There are many fluids present within the human body. Water accounts for approximately 60% of male and 55% of female body weight. Body fluids are held in two main compartments, intracellular and extracellular. Intracellular fluid is held within cells. Extracellular fluid is broken down into further categories, intravascular (plasma), interstitial, lymph, cerebrospinal and transcellular fluids (special fluids found within epithelial lines spaces). These fluids are not found in large quantities and occur in places such as the eye and ear. Nasal mucus is one of these fluids produced by goblet cells, specialised airway epithelial cells, and the submucosal glands.[25]

Figure 3 shows the breakdown of solutes between the extracellular and intracellular fluids in the human body. Taken from the book, The Fundamentals of General, Organic and Biological Chemistry.[25]

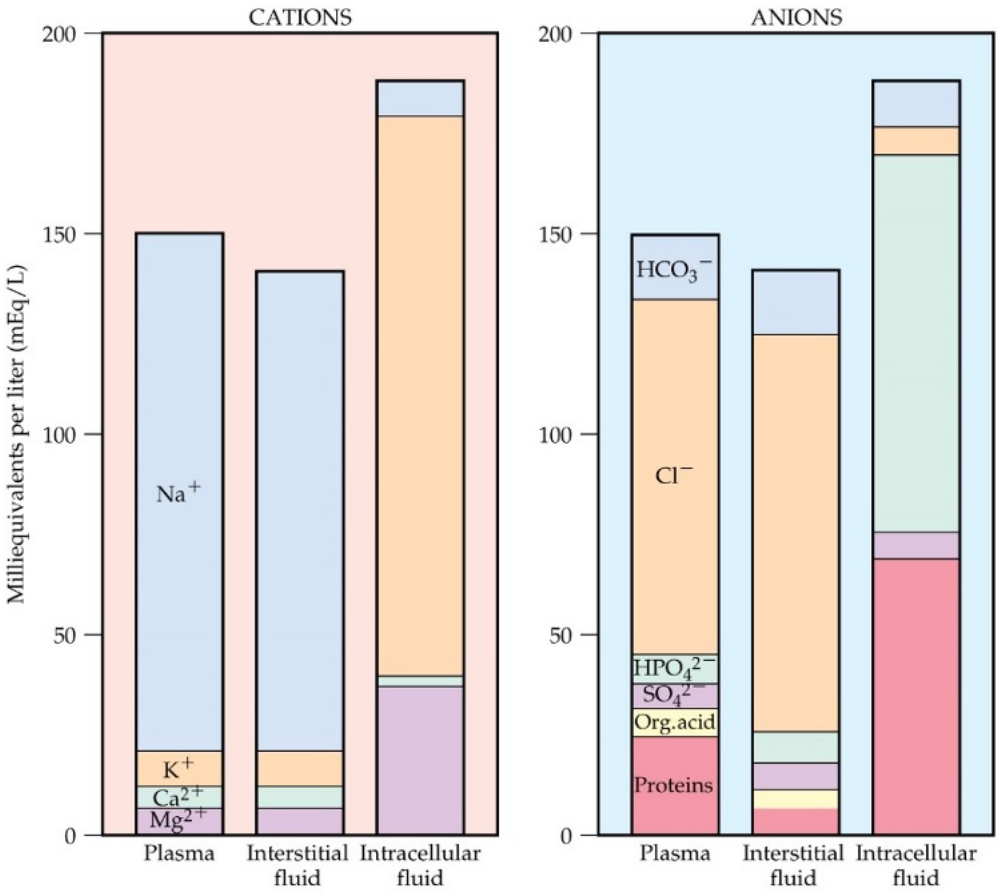


Figure 3 - Distribution of solutes between the intracellular and extracellular fluids in the human body. Taken from the book, The Fundamentals of General, Organic and Biological Chemistry.[25]

Nasal mucus is only present in small quantities in healthy subjects and is not easy to collect for analysis. It is contained in a dark narrow cavity, Figure 2, and its extraction can be invasive and painful. The collection also poses a risk of sample contamination. With blood sampling, for example, obtaining a sample is very straightforward with minimal pain, less risk of sample contamination, and is in plentiful supply. Blood has been taken, researched and analysed for over 100 years.[26] Nasal mucus has not, and there has been limited research to date. Its general composition is known, but definitive values for normal levels of electrolytes, proteins etc. are not available. Typing "composition of blood serum" into Google Scholar, 10th October 2017, finds many

papers and journals with consistent values for levels of Na^+, Ca^{2+}, K^+, other electrolytes, and proteins.

Table 1[27] gives an example of such data. Equivalent information for nasal mucus composition is simply not available.

Table 1 – Compiled from information contained in Tietz Fundamentals of Clinical Chemistry. The conventional range are the units used within the medical profession. The SI unit is the recognised unit of measurement used worldwide.

Blood, Plasma, Serum	Conventional Range	SI Range
Ca^{2+} serum	8.6 – 10.2 mg/dL	2.15 – 2.55 mmol/L
Electrolytes, serum		
Na^+	136 – 145 mEq/L	136 – 145 mmol/L
K^+	3.5 – 5.0 mEq/L	3.5 – 5.0 mmol/L
Cl^-	95 – 105 mEq/L	95 – 105 mmol/L
HCO_3^-	22 – 28 mEq/L	22 – 28 mmol/L
Mg^{2+}	1.5 – 2.0 mEq/L	0.75 – 1.0 mmol/L
Immunoglobulins, serum		
IgA	70 – 400 mg/dL	0.7 – 4.0 g/L
IgE	0 – 380 mg/dL	0 – 380 g/L
IgG	700 – 1600 mg/dL	7.0 – 16.0 g/L
IgM	40 – 230 mg/dL	0.4 – 2.3 g/L
Osmolality, serum	275 – 295 mOsmol/kg H_20	275 – 295 mOsmol/kg H_20
Proteins, serum		
Albumin	3.5 – 5.2 g/dL	35 – 52 g/L

1.3 **pH of Fluids in the Body**

The general pH of the human body is 7.2 to 7.4, but the pH of different fluids in the human body varies. **Error! Reference source not found.** gives an indication of the varying pH levels of different biological fluids.[28]

Table 2 - Indication of the pH of Biological Fluids

The pH of Some Biological Fluids	
Fluid	pH
Pancreatic Juice	7.8-8.0
Blood Plasma	7.4
Intracellular Fluids: Liver	6.9
Intracellular Fluids: Muscle	6.1
Saliva	6.35-6.85
Gastric Juice	1.25-3.0

The pH of nasal mucus ranges from 5.5 – 6.5 in normal, healthy people.[29] A more alkaline pH of 7.2 – 8.3 is found in people with a known condition such as infective or allergic rhinitis, however this pH increases with age. The mean pH of nasal mucus is thought to be 6.8 (value taken from the reference literature).[29]

1.4 **Nasal Health – Commercial Aspects**

Nasal health is of great interest to the general public. There are a variety of nasal health products on the market, from steroid based prescribed medications, to off the shelf medicated or “natural” products. Sales of cold treatments and decongestants in the UK rose by £12.5M from 2016 to 2017, with a 1.4% price rise year on year for decongestion products.[30] Consumers have been encouraged to visit pharmacies rather than the GP to relieve pressure on health services, further driving sales of these products.

One large growth area is within natural, non-medicated products, with saline, or seawater, based nasal sprays or douches.

For example, the practice of nasal irrigation (section 1.5), has been performed for centuries, with its origin dating back to an ancient Hindu ritual, which was carried out as part of their daily personal hygiene routine. They would sniff water from cupped hands and expel through the nose, a process called jala-neti, nasal cleansing.[31]

A search (December 6th, 2017) on the Boots (The Boots Company PLC) website for products targeted at nasal conditions, and available to purchase without a GP's prescription, identified 40 products including 24 seawater or saline containing items. Table 3 shows the breakdown of the primary active ingredient, or main constituent, of the products.

Table 3 - Breakdown of the primary active ingredient or main constituent of commercial products on the Boots.com website on December 6th 2017

Beclometasone dipropionate	Oxymetazoline Hydrochloride 0.05 % w/v	Xylometazoline hydrochloride 0.1% w/v.	ECTOIN 2%*	0.05% w/w fluticasone propionate	Sea Water	Saline
4	4	7	1	1	9	15
* also contains saline						

The majority of natural products on the market are saline or seawater based. Seawater or saline based products are of interest for this study. These products make claims such as, "free from steroids, drugs and preservatives", "100% micro diffused sea water", "pH balanced, buffered", "rich in trace elements and marine elements", "neutralises allergens", "prevents the common cold", and "enriched with copper", amongst others. They do not, however, list their ingredients, but only allude to them. These products are categorised as either isotonic or hypertonic solutions. Isotonic, solutions, having the same osmotic pressure as other bodily fluids such as plasma, typically are a 0.9% saline solution (9 g of salt, NaCl, per litre of water). Hypertonic, solutions having a greater osmotic pressure than other bodily fluids, are typically a 2.3% saline solution (23 g of salt, NaCl, per litre of water).

The various products claim to offer protection from the cold virus and to clear nasal congestion. A clear understanding of the modes of action are required to justify this positioning.

One of the objectives of this KESS2 sponsored research project was to consider if it is possible to produce a natural sea salt, or seawater product, which targets several nasal health issues. Would it be possible to prevent travellers from contracting the cold virus while on planes, or to prevent, or relieve, allergy symptoms? Could a targeted product treat or prevent patients developing nasal polyps,[1] or restore the correct electrolyte balance, or pH of the nasal environment? Any of these outcomes could have a positive outcome on a person's wellbeing, and if a targeted product, or range of products could be developed, there could be a positive benefit to the UK Health Service. The National Health Service is currently under increased pressure due to the COVID-19 pandemic and if a product could relieve or prevent symptoms this pressure could be reduced.

1.5 **Nasal Irrigation**

Nasal irrigation, nasal lavage or nasal douche is the process of washing the nasal passage by pouring isotonic or hypertonic saline solutions in through one nostril and out through the other. Nasal irrigation is seen as the first form of treatment in a patient with allergic rhinitis.[32] Allergic rhinitis (AR) is the most common form of non-infectious rhinitis, affecting between 10% and 30% of all adults and as many as 40% of children.[33] AR is one of the top 10 reasons for a visit to a medical practitioner.[34]

Is there any evidence to suggest that nasal irrigation, or nasal sprays containing seawater or saline solutions, have a positive effect on nasal health?

Studies have been carried out into the effectiveness of nasal irrigation and nasal sprays, but most studies do not use large numbers of participants, so their statistical value is questionable.

It is unclear how nasal irrigation works but it is implied that the action of the irrigation dislodges mucus from the lining of the nose.[35] The use of an isotonic solution has proven to be beneficial at reducing microbial antigen concentration, but reduces

lysozyme and lactoferrin levels. These levels only return to their previous state after 6 h.[36,37] Adding different ions to the solution has a positive effect e.g. Mg^{2+} promotes cell regeneration and limits inflammation,[37,38] and K^{+} has an anti-inflammatory action.[37,39] Seawater products contain these and additional minerals naturally.[40]

There have been many trials looking into the efficacy of nasal irrigation. A Cochrane review published in 2014 looked into the effects of using saline irrigation for acute respiratory tract infections which included rhinitis.[41] This review's selection criteria looked at randomised controlled trials (RCT's) and compared saline irrigation to other forms of treatment. The authors were only able to find 5 studies out of 392 that met the criteria. Many studies were not included due to the low number of subjects. The authors' view was that the use of saline irrigation did reduce the symptoms of the participants, but that due to the size of the studies, and the risk of bias, the results were not statistically meaningful.[41]

A second Cochrane systematic review published in 2016, considered saline irrigation for chronic rhinosinusitis.[42] Out of 1214 screened records only 39 full articles were screened, 2 studies were reviewed qualitatively and none were reviewed quantitatively. The 2 main studies that were reviewed were carried out by Rabago[43] and Cassandro[44] (2 out of 4 of the studies in the paper were used). Rabago[43] reported that using hypertonic saline for nasal irrigation did improve a patients quality of life, however the Cochrane review found the results inconclusive.[42]

Nasal sprays are also used as an alternative to nasal irrigation. Tano And Tand investigated the use of a saline nasal spray to prevent the symptoms of rhinitis.[45] The findings showed that the daily use of a nasal spray could help reduce the symptoms of the common cold.

1.6 **Commercial Products**

Commercial saline and seawater nasal spray products come in various forms: pressurised cans (50 – 125 mL), in the form of a salt (add approx. 3 g to 240 mL water prior to administration) and in small vertical pump action sprays (15 – 20 mL). Ten assorted salt and seawater products were selected to be analysed. These products can be seen in Table 4. Prices correct at time of purchase in December 2017.

Table 4 – The 10 commercial products selected for analysis

Product Reference	Product Name	Tonicity	Main Constituent	Volume / Mass	% Solution	Added. Minerals	Number of actuations	Price	
A	Sterimar Stop and Protect Allergy Response	Moderately Hypertonic	Seawater	20 ml	1.50%	Added Ca & Mn	120 Sprays	£8.59	
B	Sterimar Cold Defence	Isotonic	Seawater	50 ml	0.90%	Added Cu	150 Sprays	£6.80	
C	NeilMed Sinus Rinse	Isotonic	Salt		0.90%		60 Sachets	£13.69	NaCl/ sodium bicarbonate mix
D	Himalayan Salt	Isotonic	Salt	385 g	0.90%		200 Scoops	£8.54	
E	Sterimar Isotonic Nasal Hygiene Spray	Isotonic	Seawater	100 ml	0.90%		300 Sprays	£5.87	
F	NeilMed NasaMist	Isotonic	Saline	75 ml	0.90%		No data	£4.49	9 mg/ml NaCL, 1 mg/ml Sodium Bicarbonate
G	Sinomarin Hypertonic	Hypertonic	Seawater	125 ml	2.30%		No Data	£12.37	
H	Sterimar Congestion Relief	Hypertonic	Seawater	100 ml	2.30%	Added Cu & Mn	300 Sprays	£6.64	
J	NeilMed NasaMist Extra	Hypertonic	Saline	125	2.70%		No Data	£6.64	27 mg/ml NaCL, 3 mg/ml Sodium Bicarbonate
K	Sterimar Stop and Protect Cold and Sinus Relief	Hypertonic	Seawater	20	2.30%	Added Cu	120 Sprays	£8.99	

Products come in many sizes, but the products contained in the small pocket-sized vertical pump action sprays were of greater interest to this project due to their small form factor and ease of application. The 20 mL product has the capacity to deliver approximately 120 sprays at a recommended dose of 2 sprays up to 6 times per day.

The products have a shelf life in excess of 2 years from the date of manufacture and their packaging does not include a ‘throw away after x days of opening’ label. Each product contains seawater diluted with sterile water. Select products also contain additional Cu^{2+}, Mn^{2+}, Ca^{2+}, or essential oils such as eucalyptus. Products are isotonic (approx. 0.9% saline solution), moderately hypertonic (approx. 1.5% saline solution), or hypertonic (approx. 2.3% saline solution). They claim to not contain additional preservatives. The product packaging has a medicinal feel. They make claims to protect the user from the common cold, reduce symptoms due to allergies caused by pollen etc. but do not include supporting evidence on the instruction sheet contained within each box

Seawater nasal sprays (20 mL) retail for approximately £9 (approximately 7.5p per actuation), and can be purchased from pharmacies, health food stores, supermarkets and on-line retailers, such as Amazon. They are marketed as products to prevent or treat a cold or to help relieve the nasal symptoms of allergies.

1.7 **Nasal Sprays – Droplet Size and Distribution**

Whilst not the main focus of this PhD study an understanding of the nuances of some of the physical aspects of product delivery was of interest to the company partner Products come in the form of aerosols that deliver a relatively large volume, or sprays that deliver a relatively smaller volume in a fine mist. Prior research has focused on droplet size,[46,47,48,49,50] viscosity,[47] velocity,[46,49,50] distribution [47.48,49,50] and method of administration,[47.48,49,50] but not all reached the same conclusions as to their importance.

The importance of droplet size has been debated, but droplet size can be dependent upon the pressure of actuation. Inthavong *et al*.[46] developed an automated actuation system to monitor droplet size. An over the counter purchased nasal spray capable of delivering 200 doses at 50 µg per dose was used in their analysis. They found that the higher the actuation pressure, the smaller the droplets in the atomisation. They also found that the actuation pressure had a bearing on the droplet size. Adults could exert a greater pressure, giving a longer fully atomised spray with finer droplets, than the pressure that a child could exert. High speed filming was used to capture the spray plume. Figure 4[46] shows the relationship between the actuation pressure and atomisation. The greater the actuation pressure the faster and shorter the atomisation. Foo *et al*. looked at which factors made the greatest impression in nasal spray deposition.[47] They investigated the deposition patterns of the solutions through solution viscosity, surface tension, and droplet size and product administration. The deposition patterns were measured using an MRI-derived replica of a nasal cavity. A fluorescent marker, rhodamine 590, was used for detection. The research analysed spray plume angles between 29 - 80°, with droplet sizes ranging from 37 - 157µm. All measurements were recorded at administration angles 30°, 40° and 50° above horizontal to look at how the administration angle affected deposition. The quantity of spray deposited was determined by measuring the amount of rhodamine 590 in each section of the disassembled replica nose.

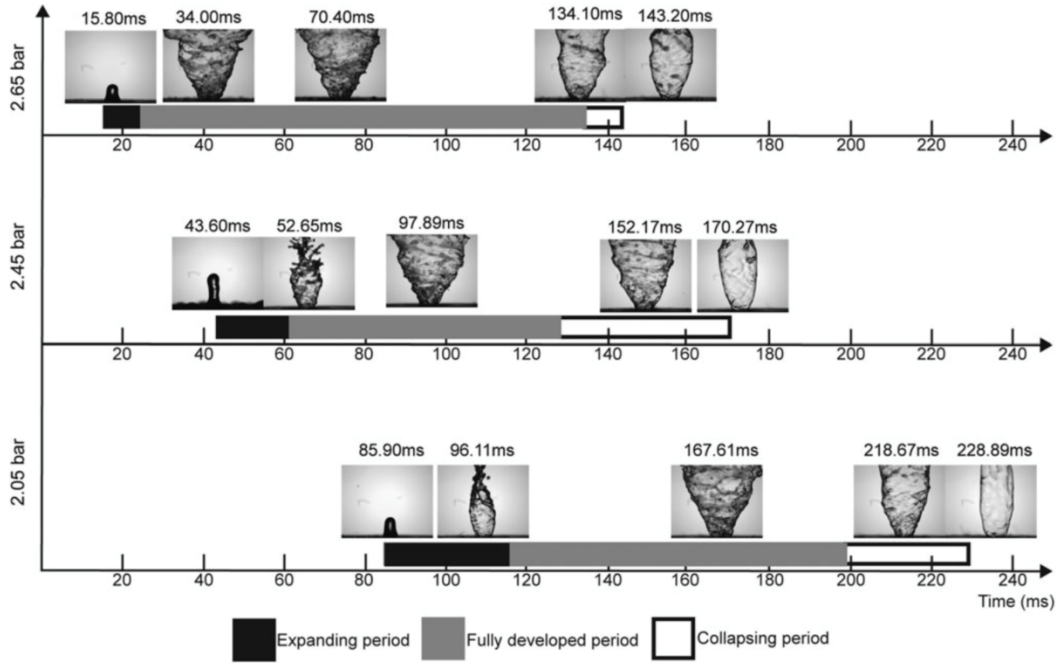


Figure 4 - Spray plume development displaying the relationship between actuation pressure and atomisation[46] Image used with the permission of the author

The results show that both plume angle and administration angles are important factors in deposition. This data can be seen in Table 5.

Table 5 - Plume and administration angle distribution results complied from Foo et al.'s [47] research. The table shows the administration and plume angles necessary to obtain the maximum turbinate coverage.

Administration Angle	Plume Angle	Turbinate Distribution
40-50°	55-65°	30-50%
30°	55-65°	75%
30°	<30°	90%

All other factors including droplet size had only minor influences on deposition.

Cheng *et al*. developed a multi sectional model of the nasal airway using an *in vivo* magnetic resonance image (MRI) scan of an adult male to investigate deposition of nasal sprays.[48] The replica nasal model was constructed from 77 acrylic plastic sections of 1.5 mm in width. The research found aerosols deposited in the anterior and turbinate regions, with little spray reaching the nasopharyngeal region. Deposition

was found to be high towards the anterior portion, with the highest levels seen on the inferior meatus. Figure 5 shows a re-drawing of the test system used by Cheng *et al*. to determine spray deposition.[48]

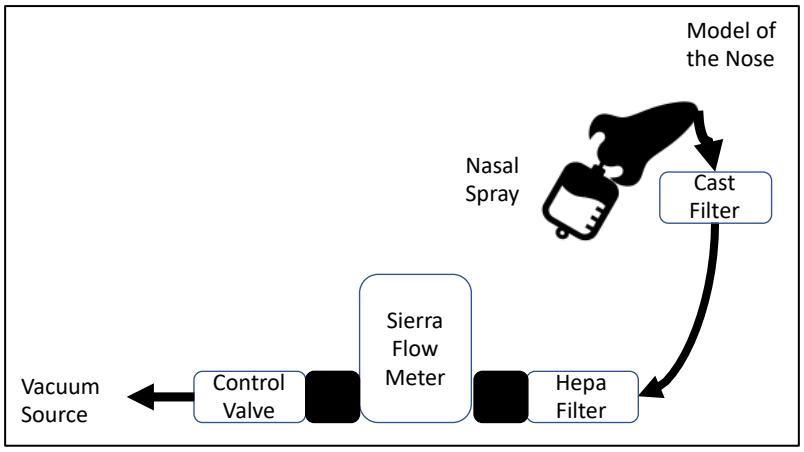


Figure 5 – Drawing of the test system used to determine spray pattern deposition, from Cheng et al.

Laser diffraction was used to analyse droplet size while high speed photography captured the spray angle. The results indicated a correlation between droplet size, the spray angle and deposition in the anterior region of the nasal airway. A larger droplet size with a wide spray angle increased deposition, and this prevented deposition in the turbinate region.

Tong *et al*. used a computerised model of realistic nasal cavity from a CT scan of a healthy male to investigate the effects of the delivery device and the orientation of the spray nozzle.[49] The actuation force of an adult was simulated to produce a representative spray plume. A middle spray direction was shown to give the best spray efficiency when compared to upper or lower directions using a particle size of 10 µm.

Inthavong *et al*. used Computational Fluid Dynamics (CFD) software to optimise nasal spray parameters.[50] Their aim was to use CFD to gain insight into the variables controlled by the consumer and the parameters that these affected. These can be seen in Table 6.[50]

Table 6 - Variables related to the actuation of a nasal spray

Consumer variables	Parameter affected
Increased/decreased inhalation	Airflow rate
Nostrils opened (one or two)	Airflow pattern
Head tilt back/forward	Insertion angle inwards
Spraying away from septum walls	Insertion angle sideways
Strength of actuation	Particle size and velocity
Speed of actuation	Particle size and velocity
Insertion location	Surrounding geometry

The research found that the important parameters in a nasal spray were particle size, the diameter of the spray cone at break up length, and the spray cone diameter.

All of the research is varied, and no two pieces of research totally agree on the parameters. Research does indicate that higher pressure and greater speed of actuation, coupled with a lower angle of actuation and lower plume angle give the greatest deposition within the nasal cavity.[47]

1.8 Collection Methods and Analysis of Nasal Mucus

Collection of blood for analytical and diagnostic purposes has been common for many years but the same cannot be said for nasal mucus. The general composition of nasal mucus is known but has had a limited amount of study. For example, it is not understood if the electrolyte balance plays a part in overall nasal health.

To date, there is not a reliable, reproducible, collection method available to collect nasal mucus for analysis, or to aid in the diagnosis of conditions affecting the nose. Various approaches that have been published are described below.

Filter paper techniques were employed in studies by Lorin[4] & Knowles.[5 51] Lorin *et al.*[4] used strips of Whatman number 50 filter paper cut into strips, 5 x 55 mm in size, washed in deionised water, and dried at 90 degrees Celsius in a muffle furnace overnight. Before use, the filter paper was acclimatised at room temperature for 24 h and tested to ensure negative for electrolytes or proteins.

Nasal mucus samples were collected by insertion of the filter paper 1.5 – 3.0 cm into the nose, parallel to the septum, at an angle of 30 – 60 degrees with the floor of the nose. After 10 s the wet portion of the filter paper would be cut and placed in the sample vial. This would be repeated until the filter paper had all been used. The process would be repeated on the second nostril the following day for protein samples. The vials were reweighed, and volumes calculated. The filter paper was left to soak in the eluent for up to 24 h before the eluate was pipetted out. Na^+, K^+ and Ca^{2+} levels were measured via atomic absorption spectroscopy (AAS). Flame photometry was also used to measure K^+ concentration. Albumin and immunoglobulins were measured by single gel diffusion.

Ruocco *et al.* proposed a different collection method to evaluate nasal mucus proteins.[6] This involved using pieces of gauze, 1 mm in thickness and 1 x 1 cm^2 in size, cut from a square of pre-humidified (with 10 µL of $H_2PO_4^-$ buffered saline (PBS)) gauze. Samples were taken early in the morning from a seated subject with their head tilted backwards at an angle of 30°. The gauze was passed between the inferior turbinate and the septum for 10 s. Two samples were taken from the nasal cavity. The gauze, containing the mucus sample, was placed in a plastic tip which was placed in a test tube with 200 µL of PBS, pH 7.4. The samples were centrifuged at 2000 *g* for 20 min at a constant 15 °C, before being collected in pre-weighed plastic sample tubes. Samples were stored at -20 °C and total protein was established via the Coomassie Method.[52] Coomassie Brilliant Blue G-250 binds to the protein in the sample, and causes a change in absorbance from 465 to 595 nm There was no attempt to analyse the electrolytes in this study, however, it is feasible that this collection method could be used to obtain samples for cation and anion analysis.

In the study by Knowles *et al.*, samples were taken from healthy subjects to compare electrolyte concentrations with those with a diagnosis of cystic fibrosis,[5] an inherited condition which affects the movement of salt and water between cells, causing sticky mucus to build up in the lungs and other organs. A filter paper technique which had previously been assessed by Knowles was used.[51] To enable collection, pieces of Whatman 541 hardened ashless filter paper were cut into strips, 4 cm in length by 5 mm in width. The filter paper was washed three times in double-distilled deionized water (DDH_2O) prior to being dried in an oven. On the day of sampling, prior to use,

the filter paper was folded in half width ways and the weight noted. The sample was obtained by placing the filter paper sideways in a V shape under the inferior turbinate, using a hemostat and left in place for 20 s. Measurements were taken every 15 s for 90 s after removal. Samples were analysed in several ways. For the analysis of anions and cations. 1 mL of DDH_2O was added to the sample and agitated for 24 h in a gyratory shaker. The solution in the vial was transferred to an acid washed volumetric tube and 0.5 mL of 0.2M HNO_3 added to the sample vial. The filter paper was rung out using forceps and the extract added to the volumetric tube. The procedure was repeated, and the volume of the extracted liquid was made up to 2 mL with 0.2M HNO_3. Chloride ions were determined using a chloridometer. Na^+ and K^+ were analysed via flame photometry. To establish the albumin content, filter papers were soaked overnight in 200 mL of DDH_2O and analysis determined *via* Radioimmunoassay. Osmolality was determined by a freezing point osmometer.

A variety of techniques were employed by Vanthanouvong and Roomans[8] to collect and sample nasal mucus. These included filter paper, cotton wool, direct collection with a pipette and filter paper with Sephadex beads, Figure 6, prior to being analysed via X-ray microanalysis.

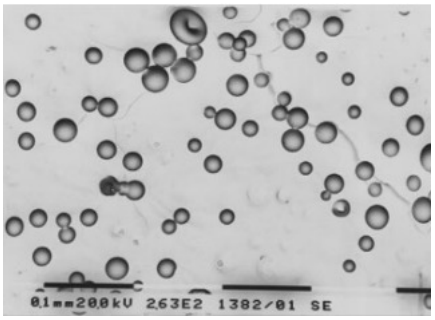


Figure 6 - Low-power scanning electron micrograph of Sephadex G-25 beads mounted on tape and filter paper used to collect nasal fluid

Samples collected *via* direct pipetting from the nose, were placed on to either filter paper or onto or carbon planchets. Samples were also collected by the insertion of filter paper into the nostril, (3 x 3 mm strips), and being left in position in the vestibule of the nose for 1 min. The filter papers were dried at room temperature and coated with a carbon conductive layer. Prior to collection via cotton wool (7 mg), the subjects' nostrils were held closed for 10 min before insertion.

On insertion, the subject held their breath for approximately 30 s. On removal, the cotton wool was placed in a in a micropipette tip and centrifuged, (4,000 *g* for 2 min), to remove the liquid phase. The liquid component of the nasal mucus sample was collected via a micropipette and 1 µL placed on either a piece of filter paper or on a carbon planchet. The cotton wool, still containing mucus, was also dried and coated in a conductive carbon layer. Samples were analysed under an electron microscope. Another method employed involved the use of filter paper with Sephadex beads.[53] These beads can separate low and high molecular weight molecules. The beads were taped to Whatman filter paper and inserted in the nostril for 10 min. After treatment with hydrophobic volatile silicon oil to remove all fluid, the beads were separated and placed on a nylon electron microscopy grid for analysis.

Burke[10] looked at the ionic composition of nasal fluid, by collecting samples of his own nasal mucus in several different ways. Collections were made using a small metal or plastic spoon and samples were placed into a 1.5 mL microcentrifuge tube immediately. Collecting mucus from a sneeze was facilitated by utilising a stainless-steel device clipped onto the septum of the nose, the secretion then collected in a microtube. Samples were also collected spontaneously (with no stimulant), at cold temperatures (~4 ^{O}C), and from secretions of a common cold. All samples were collected from the nose except for collections at ~4 ^{O}C, which were taken from the mouth. A total of 21 samples were collected with all samples being at least 0.25 mL in size. Na^+, K^+ and Cl^- were analysed using ion-selective electrodes. The measurements of HCO_3^-, urea, Ca^{2+}, Mg^{2+} and $H_2PO_4^-$ were carried out using an Aeroset chemistry analyser, an automated random-access clinical chemistry system.

Henkin *et al.* took spontaneous discharge samples from the subjects' nares first thing in the morning.[9] This was to avoid any contamination from the collection medium. Samples were taken from 18 subjects, 7 with no history of nasal problems and 11 with a history of nasal disease. This study primarily focussed on protein and enzyme determination, but also carried out analysis for the trace metals Zn^{2+}, Cu^{2+}, Mg^{2+} and Ca^{2+} using atomic absorption spectroscopy.[54]

Obtaining nasal mucus samples through nasal washing is another technique that has been employed.[3,55] In 1964 Remmington *et al.* looked at 2 differing groups of

volunteers, normal and allergic, the allergic category having a history of nasal allergy but showing no outward symptoms on the day of collection. The collection method was adapted from the method used by Anderson *et al*.[55] Where 5 mL of 0.15 M NaCl replaced 5 mL of distilled water, this was found to cause less irritation in the washing process. The solution was passed through each nostril in turn while the head was tilted, and the resultant secretions collected in a sterile Petri dish held below the nose. This study focussed on protein determination and did not analyse the samples for ionic composition. This is a qualitative method and gives a view of proteins, antibodies, electrolytes etc. present, but it would be difficult to ascertain quantitative concentrations due to dilution with nasal washing.

Narkowicz *et al.* looked at the effect of tobacco in smokers, non-smokers and passive smokers by collecting nasal mucus in sample vials.[21] Nasal mucus was collected from 53 healthy subjects between the ages 20 – 30 years of age. Subjects were asked to blow their nose into a sterile plastic vial. Samples were prepared using 2 mL de-ionised water and analysed for the cations Na^+, K^+, Mg^{2+}, NH_4^+ and Ca^{2+}, and also for the anions F^-, Br^-, Cl^-, NO_3^- and thiocyanate using ion chromatography. The main focus of this study was on the thiocyanate ions. The participants were split into non-smokers, active smokers and passive smokers. This method is a reproducible way of collecting mucus samples from participants but may prove to be unsuitable in a clinical setting for participants that have trouble clearing their nasal passages by blowing their nose.

Gröger *et al.* utilised cotton wool pellets as the collection medium for nasal mucus.[56] The mucus of 120 patients, split into 4 groups, was analysed alongside blood samples. This study was looking at eosinophils and mast cells and their links to allergic rhinitis and nasal polyps. The size of the cotton wool pellets in this study and the volume of mucus obtained were not recorded. The solubilisation technique used in Gröger's study involved the use of PBS. This is not an option when needing to quantify concentrations of Na^+.

Comparing the results from these studies is not straightforward as there are no common approaches. In each, the participants, methods and modes of collection vary.

There are many issues arising from this body of research. There are very few participants in their sample sets. It is unclear how each of the researchers categorise

their healthy participants. The pathological participants have a range of medical illnesses from cystic fibrosis, to nasal polyps, and chronic rhinitis. These conditions may have different electrolyte concentration values to one another. It is unclear if all of the studies collected mucus from the same area of the nose. Different collection matrices and analytical techniques have been used. The accuracy of measurements or the equipment could impact on the results.

This data gives a qualitative view, reproducing the data could prove problematic. **Error! Reference source not found.** displays results of previous research.

Table 7 - Results of previous studies carried out by Vanthnaouvong & Roomins,[7,8] Lorin,[4] Knowles,[5] Burke,[10] Henkin[9] & Narkowicz[21]

Ion	Study A	Study B	Study C	Study D	Study E	Study F	Study G	Study H
	mM (Mean + SD)	mM (Mean + SD)	mM (Mean + SD)	mM (Mean + SD)	mM - (approx Value)	mM (Mean + SEM)	mM (Mean + SD)*	mM (Mean + SD)**
Na^+	127 ± 6	142 ± 28	141 ± 8	150 ± 32	110	184 ± 37		74.5 ± 36.2
K^+	27 ± 3	43 ± 10	61 ± 8	41 ± 18	30	32.6 ± 5.2		14.4 ±7
Ca^{2+}	5 ± 1			4 ± 2		1.5 ± 0.27	1.33 ± 0.08	2.2 ± 3.1
Mg^{2+}						0.72 ± 0.21	0.65± 0.08	0.41 ± 1.03
Zn^{2+}							0.002	
Cu^{2+}							0.002	
Cl^-	140 ± 7	150 ± 36	170 ± 12		125	217.5 ± 56.2		
HCO_3^-						10 ± 2		
$H_2PO_4^-$				0.7 ± 1.5		0.73 ± 0		
No of healthy Participants	8	8	19	10	8	2	7	14
No of Pathalogical Participants	3	3	0	0	9	0	11	0

A[8] = Vanthnaouvong & Roomins (2004) - Collected with a micropipette
B[8] = Vanthnaouvong & Roomins (2004) - Collected with a Sephadex G-25 ion exchange beads, mounted on tape, applied to filter paper
C[7] = Vanthnaouvong & Roomins (2006) - Collected with a Sephadex G-25 ion exchange beads, mounted on tape, applied to filter paper (results only for healthy participants)
D[4] = Lorin *et al* (2004) Using Filter Paper
E[5] = Knowles *et al* (2000)
F[10] = Burke (2004) - using spontaneous collection method
G[9] = Henkin *et al* (2000)* not for Zinc or copper
H[21] = Narkowicz *et al* (2013)** Results for non-smokers only

The numbers of participants in each of the studies is too small to make the findings statistically meaningful. For example, in study F[10] only 2 samples were used, both from the author. The results are given using the mean ± the standard error of the mean

(SEM), giving an expected spread of results should further samples have been used. This will not give a representative view of the general population. Using an approximate calculation[10] for the margin of error $^{1}/_{\sqrt{N}}$ where N is equal to the sample size, the margin of error could be in the region of 70%. The participant numbers in all studies are too small to give a quantitative view, but the results fall in a comparable range giving a consistent indication as to ion concentration. Study H,[21] 25 active,14 non-smokers and 14 passive smokers nasal mucus was analysed. It is unclear whether the participants were healthy or suffered from any nasal conditions.

Across the range of studies the value for Na^+ concentration varies, but allowing for the standard deviation values, it falls close to the known range for blood serum (136 – 145 mM) as seen in Figure 7.[27]

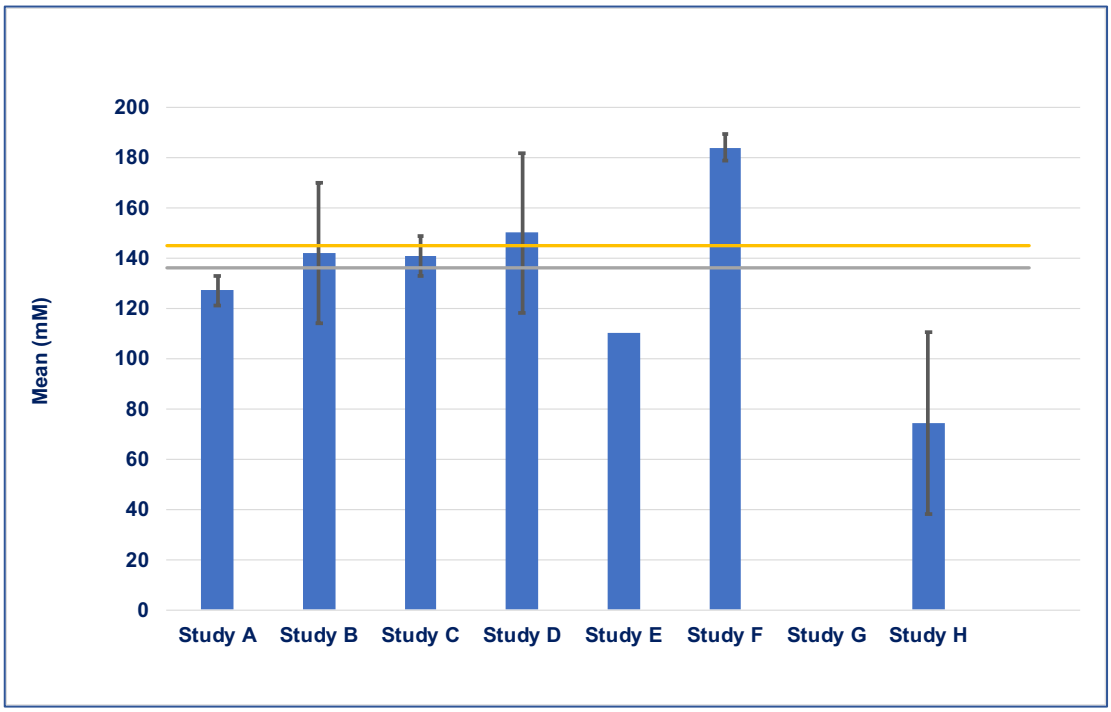


Figure 7 - The Na^+ concentration in each study in comparison to known value in blood plasma (lines showing the upper and lower range in mM for blood plasma) compiled from information contained in Tietz Fundamentals of Clinical Chemistry

This data indicates that the Na^+ concentration in nasal mucus is approximately the same as blood plasma, whereas K^+ levels are considerably higher. The study by Knowles *et al.* finds that Na^+ concentration is approximately 25% lower than that of

plasma.[5] This disagrees with the findings of the other studies. Figure 8 displays the comparison of K^+ in blood plasma and nasal mucus studies to date.

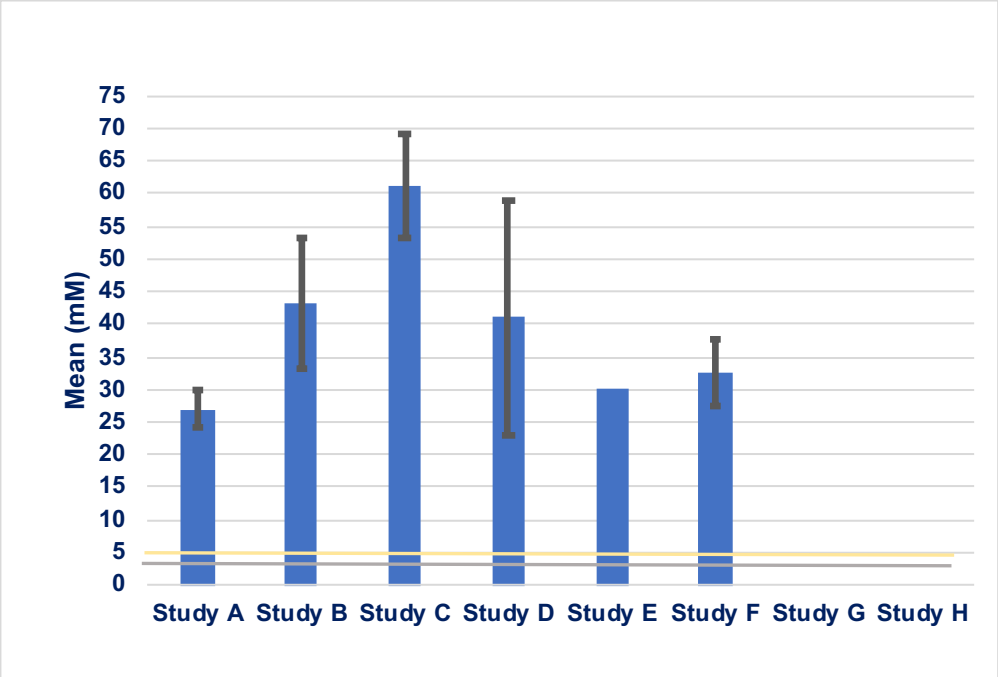


Figure 8 - The K^+ concentration in each study in comparison to known value in blood plasma (lines showing the upper and lower range in mM for blood plasma) compiled from information contained in Tietz Fundamentals of Clinical Chemistry

Participants are considered healthy if they do not have a history of nasal disease or any outward symptoms; this is consistent across all studies. Lorin[4] and Burke[10] only used healthy participants. Vanthanounong *et al*.[7] also looked at participants with cystic fibrosis (CF) and other nasal conditions. Female CF participants had higher levels of Na^+, K^+, and Cl^- than their male counterparts, and females with severe symptoms generally had higher levels than females with mild symptoms. This would indicate that there is an increased electrolyte concentration with nasal diseases and CF, and this increases further with severity. This is contradicted in the research from Knowles et *al*.[5] This study found no significant difference between groups.

Different collection matrices have been employed in the studies and where the same matrix has been used (e.g. filter paper [4,5,51]), the collection method and the analytical techniques used have differed, as have the results. It is difficult to assess the effectiveness or reproducibility of each method, or to understand the experience of the participant. Did they find the procedure invasive? Was it painful? How easy was it to

obtain a sample? Could any of these methods be used as a routine procedure to test for electrolyte concentration in a clinical setting?

Current research gives a baseline for further investigations. At present it is unclear how electrolyte concentrations vary in healthy and pathological groups or what impact variances in electrolyte concentration levels affect nasal health.

2 Techniques

2.1 Ion Chromatography

Ion chromatography[57] (IC) is a separation technique and a class of liquid chromatography. It uses liquid as the mobile phase and packing inside a column as the stationary phase. Ionic species are separated based on their interaction with a resin. Sample solutions pass through a pressurized chromatographic column where the ions are absorbed by column constituents. Eluent, an extraction liquid, runs through the column. The absorbed ions separate from the column based on their size and type. Different ions have different retention times on the column. Figure 9 shows the basic cation process.

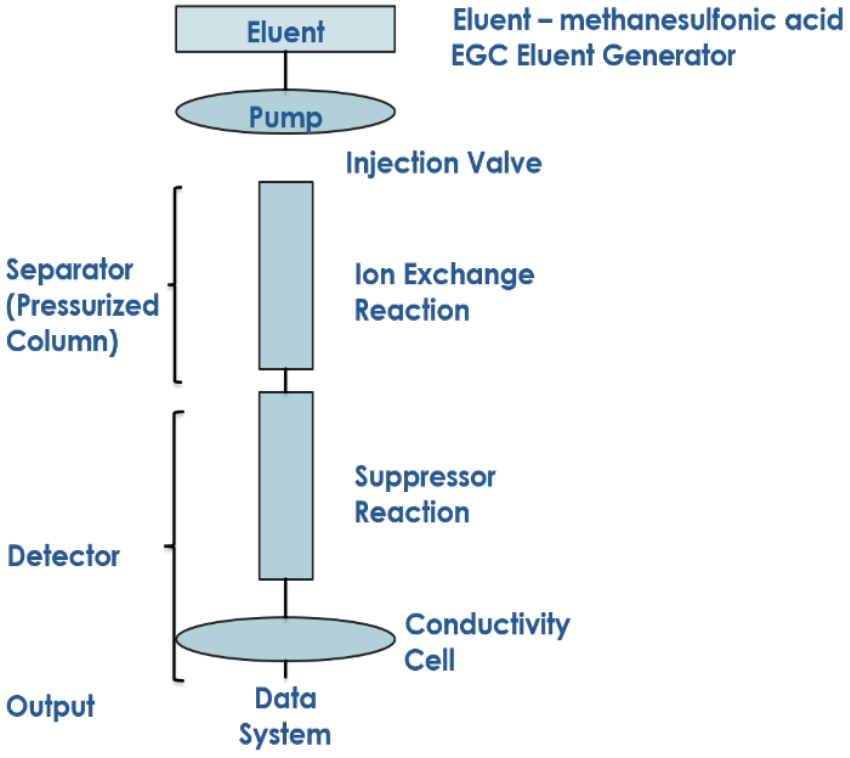


Figure 9 - Ion chromatography cation separation process flow

2.2 Inductively Coupled Plasma – Optical Emission Spectroscopy

Inductively coupled plasma – optical emission spectroscopy (ICP-OES) is a technique used in the analysis of trace elements in solution.[58] The emission spectra of a sample are used to identify and quantify which elements are present. Samples are de-solvated, ionised, and excited by the plasma. The elements are identified by their emission lines and the intensity quantifies the concentration with calibration standards. Ca^{2+} is measured at two different wavelengths as Ca^{2+} can be prone to self-absorption if high concentrations are present. Figure 10 shows the simplified process.

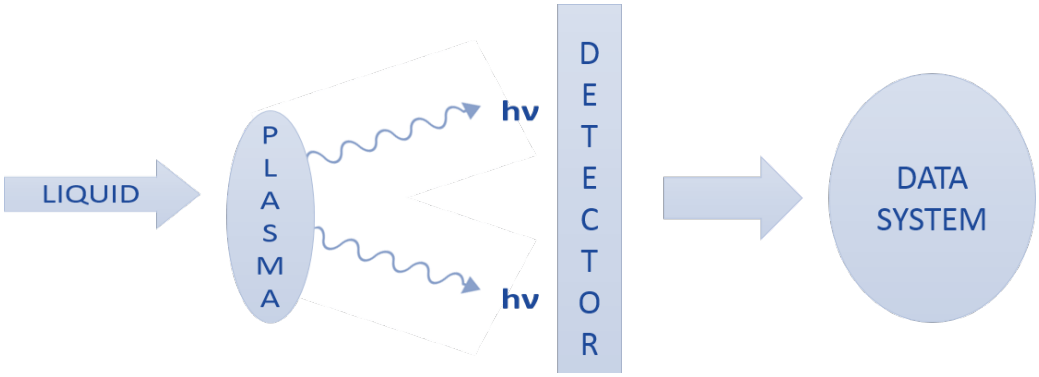


Figure 10 - Simple depiction of ICP-OES

The ICP-OES can run samples with calibration standards, or it can run in a semi-quantitative mode. In semi-quantitative mode, the upper level of detection is 5 mg/L, with a lower limit of 10 µg/L.[59]

2.3 Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)

ICP-MS is a technique used in the separation of trace metal elements in solution.[60] ICP-MS can detect low level concentrations in the ppb (µg/L) or ultra-low concentrations in the ppt (ng/L) range. Atomic elements such as Cu, Mn and Zn are passed through an Ar plasma source, that can reach temperatures of up to 10000 K, and are ionised. The ions are then sorted according to their weight. This technique has the advantage that it allows for very low-level measurements, but there can be interference issues with the argon gas. This causes problems with elements such as Fe, Mn and As, but can be overcome by analysing the samples in He mode.[61] Figure 11 show the workings of an ICP-MS and Figure 12 an Agilent 7700 ICP-MS.[62]

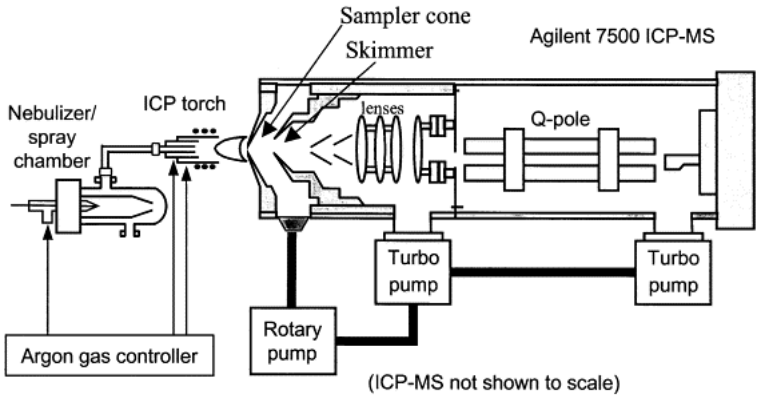


Figure 11 - ICP-MS Schematic diagram[62]

Figure 12 – Agilent 7700x ICP-MS

2.4 **Atomic Absorption Spectroscopy (AAS)**

AAS,[63] is a spectroscopic technique used to determine the concentration of elements in a sample. The sample is atomised in a flame. The flame reaches a temperature of 2300 °C using air and acetylene and 2700 °C using N_2O and acetylene. Atoms of different elements are absorbed at different wavelengths using a $h\upsilon$ source made from the same element. Figure 13 shows a simple depiction of the AAS process.

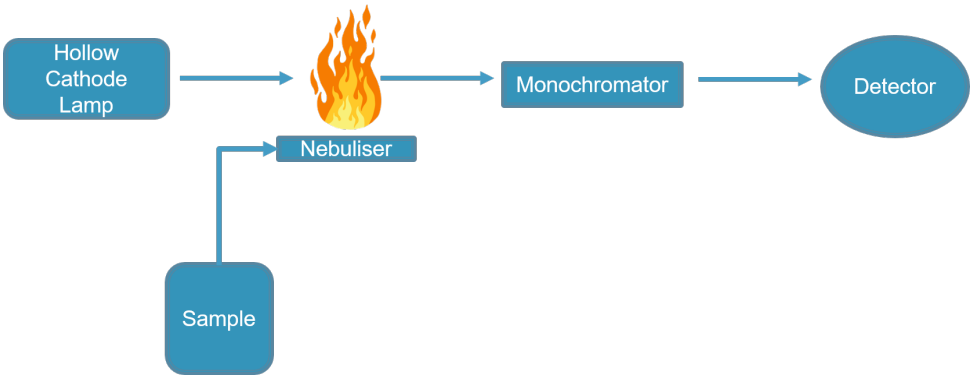


Figure 13 - Simple depiction of AAS process flow

A hollow cathode lamp is used as the light source. This consists of a W anode and a cylindrical hollow cathode made of the element being analysed, *e.g.,* a Cu cathode when determining Cu. The anode and cathode are contained in a glass tube filled with an inert gas. Figure 14 shows a hollow cathode lamp.

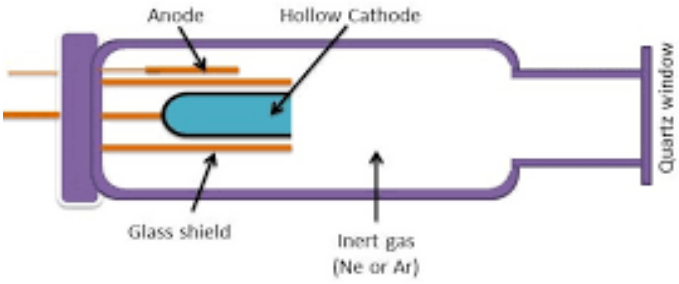


Figure 14 - Hollow cathode lamp

2.5 **Osmolality Testing**

Osmolality is the measure of the number of particles dissolved in a kg of fluid. Osmolality is a typical test for characterising bodily fluids. A normal level in serum falls between 275-299 mOsm/kg. Isotonic solutions should be close to, or within this range. The osmolality or the concentration of the sample is determined by the freezing point; the greater the concentration, the lower the freezing point. Osmolality testing was performed in the clinical chemistry laboratory at Ysbyty Gwynedd, Bangor, using an Advanced Systems Osmometer 3320 freezing point osmometer as seen in Figure 15.

Figure 15 – Advanced Instruments freezing point osmometer model 3320

2.6 **pH Measurements**

pH testing or potential of hydrogen will be performed to assess the acidity and alkalinity of the commercial products. Due to the solubilisation methods and the viscosity of the nasal mucus samples it will not be possible to measure the pH of the mucus. The pH scale is a logarithmic scale defined as $-\log_{10}[H^+]$.[64] [65]

3 Analysis of Existing Commercial Products

Sea water and saltwater based nasal wash products were purchased from an online retailer and a local pharmacy. These products are sold as allergy and cold relievers. The aim of this study was to determine the cation composition of the 10 products seen in Table 8. The products were a mix of isotonic[66] and hypertonic[67] solutions. The aim was to investigate if there was a correlation between the cation concentrations in the nasal wash products and those found in nasal mucus. This, in turn, forms part of the investigation into the feasibility of producing a range of new sea water nasal products. Such products might be used as a daily health nasal spray or to prevent or treat a range of nasal illnesses.

All seawater products are produced using seawater from Cancale Bay in Brittany. Several analytical techniques were employed due to the composition of the nasal wash products. This included ion chromatography (IC), inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectroscopy (AAS) osmolality and analysis of the pH of the products. Of the 10 commercial products selected, 5 were labelled isotonic (B, C, D, E and F), 4 hypertonic (G, H, J and K) and 1 product (A), did not give an indication as to whether it was isotonic or hypertonic. It was treated as isotonic for the purposes of dilution for analysis.

Table 8 – The 10 over the counter commercial products purchased for analysis from a high street retailer

Product ID	Nasal Product	Categorisarion	Hypertonic/Isotonic
Product A	Sterimar Stop and Protect Allergy Response	Seawater based	Moderately Hypertonic
Product B	Sterimar Cold Defence	Seawater based	Isotonic
Product C	NeilMed Sinus Rinse	Saline Based	Isotonic
Product D	Himalayan Salt	Saline Based	Isotonic
Product E	Sterimar Isotonic Nasal Hygiene Spray	Seawater based	Isotonic
Product F	NeilMed NasaMist	Saline Based	Isotonic
Product G	Sinomarin Hypertonic	Seawater based	Hypertonic
Product H	Sterimar Congestion Relief	Seawater based	Hypertonic
Product J	NeilMed NasaMist Extra	Saline Based	Hypertonic
Product K	Sterimar Stop and Protect Cold and Sinus Re	Seawater based	Hypertonic

3.1 Results and Discussion, IC – Commercial Products

3.1.1 Exploratory Nasal Wash Sample Bulk Metal Analysis

In order to gauge the sensitivity of the Dionex ICS-2100 ion chromatography machine (IC), one product was selected for exploratory analysis. Dilute samples of an isotonic nasal spray were analysed.[66] Isotonic nasal sprays are typically a 0.9% saline solution, meaning there are 9 g of sodium chloride (NaCl) in 1 litre giving a Na^+ concentration of approximately 3.5 g/L. Based on this number, a stock solution of approximately 200 mg/L was made, from which, diluted samples ranging from approximately 1 mg/L to 200 mg/L were prepared.

Initially one isotonic product (B) was selected to give an indication of ionic strength[68] in the commercial products. Retention times corresponding to Na^+, K^+, Mg^{2+} and Ca^{2+} were recorded Table 9 shows the calibration data.

Table 9 - Data for Na^+, K^+, Mg^{2+} and Ca^{2+} for a linear calibration curve, detailing the gradient and the calibration coefficient value

Calibration Summary							
Peak Name	Eval.Type	Cal.Type	Points	Offset (C0)	Slope (C1)	Curve (C2)	Coeff.Det. %
Na^+	Area	Lin	18	0	0.102	0	99.27%
K^+	Area	Lin	17	0	0.066	0	99.16%
Mg^{2+}	Area	Lin	18	0	0.182	0	99.19%
Ca^{2+}	Area	Lin	18	0	0.117	0	99.15%

Table 10 gives a qualitative view of the cation concentration in an isotonic nasal spray product.

Table 10 - Concentration data for Na^+, K^+, Mg^{2+} and Ca^{2+} showing the retention time, peak area, peak height and concentrations

Time (Mins)	Peak Name	Area µS*min	Height µS	Amount mg/L	Concentration of Product mg/L	NaCl Concentration mg/L	% NaCl Solution
3.71	Na^+	16.461	86.56	161.71	2862	7275	0.7
4.83	K^+	0.302	1.553	4.5751	81	N/A	N/A
7.64	Mg^{2+}	3.493	4.348	19.23	340	N/A	N/A
9.25	Ca^{2+}	0.78	0.845	6.6612	118	N/A	N/A

This gave values equivalent to a 0.7% saline solution, lower than expected. The product packaging indicated that the product was isotonic. These results were used to give a concentration to enable preparation of all 10 commercial products for cation analysis.

3.1.2 **Nasal Spray 10 Product Initial Bulk Metal Analysis.**

All products were initially diluted to approximately 100 ppm. Two of the products were dry salt based (C and D), nasal irrigation products that required mixing with water prior to use. See section 3.8. Two different batches of each product were used. The calibration data is displayed in Table 11. The calibration curves can be seen in Appendix C

Table 11 – Calibration data IC analysis 2 for Na^+, K^+, Mg^{2+} and Ca^{2+}

Ion	Ret Time	R^2	Point 1 (mg/L)	Point 2 (mg/L)	Point 3 (mg/L)	Point 4 (mg/L)	Point 5 (mg/L)	Point 6 (mg/L)
Na^+	3.740	99.759	5	10	20	50	75	100
K^+	4.823	99.962	12.5	25	50	125	187.5	250
Mg^{2+}	7.477	99.985	6.25	12.5	25	62.5	93.75	125
Ca^{2+}	8.997	99.986	12.5	25	50	125	187.5	250

The limit of detection (LOD) and limit of quantification (LOQ) were calculated for Na+ using the readings for the blank (HPLC grade water). The LOD was 2.9 mg/L (the mean of the blank plus 3 standard deviations)[69] with the LOQ 4.1 mg/L (the mean of the blank plus 10 standard deviations.)[69] It was not possible to calculate the LOD or LOQ for the other ions present as these were not detected in the blank and there were insufficient low concentration replicates. The chromatograms in **Error! Reference source not found.** and Figure 17 show the concentration of the cations in the commercial products. It was not possible to resolve the peaks for Ca^{2+} and Mg^{2+} under the conditions used. K^+, Mg^{2+}, and Ca^{2+} are not present in the NeilMed products (NasaMist, Nasal rinse or Himalayan salt). It was expected to see concentrations of K^+, Mg^{2+} and Ca^{2+} in seawater products, the packaging indicates that they are produced from 100% diluted seawater.[70]

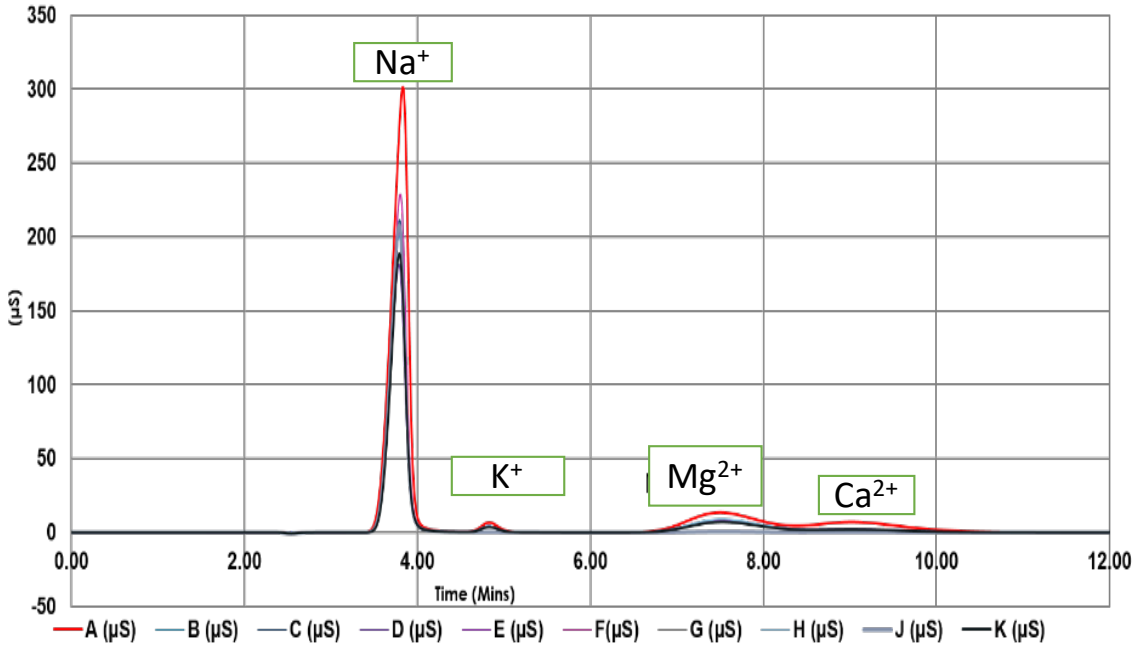


Figure 16 - Overlaid chromatogram for Na^+, K^+, Mg^{2+} and Ca^{2+}

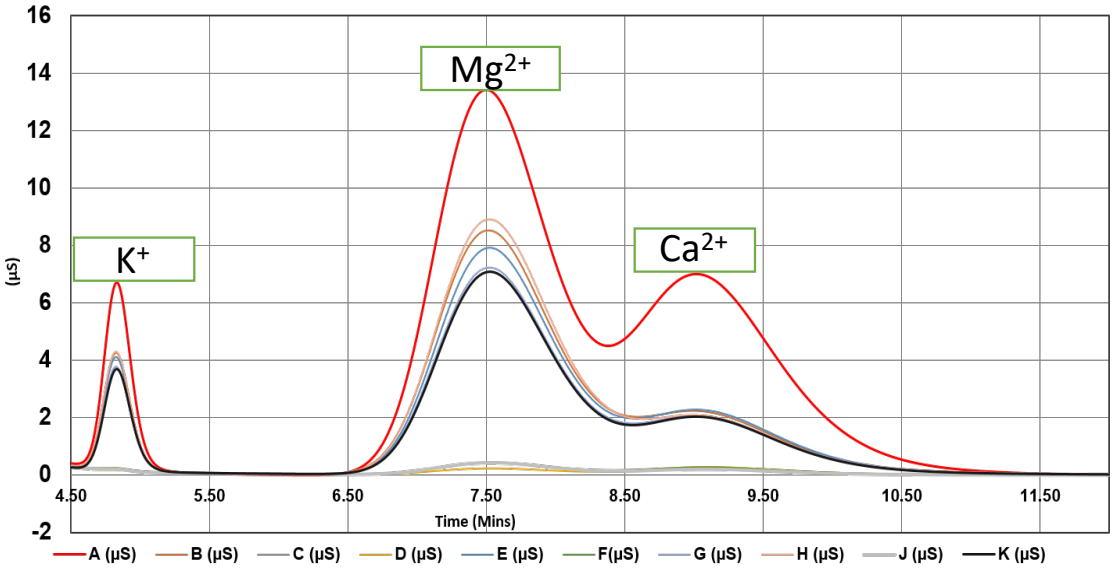


Figure 17 - Expanded chromatogram for K^+, Mg^{2+} and Ca^{2+}

Table 12 displays the mean cationic concentration for products A-K.

The results in Table 12 show products B, C, D, E, and F, are isotonic (a range of 3420 to 3780 mg/L Na^+ concentration), while G, H, J, and K, are hypertonic (a range of 8426

to 9915 mg/L of Na^+). Product A has a higher Na^+ concentration than is normally seen in an isotonic solution but is lower than expected for a hypertonic solution, but it would still classify as hypertonic.

Table 12 - Mean concentrations for Na^+, K^+, Mg^{2+} and Ca^{2+} ions present in the 10 products as determined by IC

	Na^+ Concentration (mean) mg/L	Std Dev	K^+ Concentration (mean) mg/L	Std Dev	Mg^{2+} Concentration (mean) mg/L	Std Dev	Ca^{2+} Concentration (mean) mg/L	Std Dev
Product A	5948	375	166	28	686	59	619	79
Product B	3735	216	132	55	432	76	141	67
Product C	3780	197	0	0	0	0	0	0
Product D	3420	213	0	0	0	0	0	0
Product E	3586	110	106	10	416	24	172	32
Product F	3719	167	0	0	0	0	0	0
Product G	8426	433	260	11	959	65	391	56
Product H	9207	632	283	20	1120	83	397	38
Product J	9915	755	0	0	0	0	0	0
Product K	8531	380	250	20	973	58	391	55

Figure 18 shows the % saline solution for each product and the expected levels for isotonic and hypertonic solutions. The error bars indicate the standard deviation.

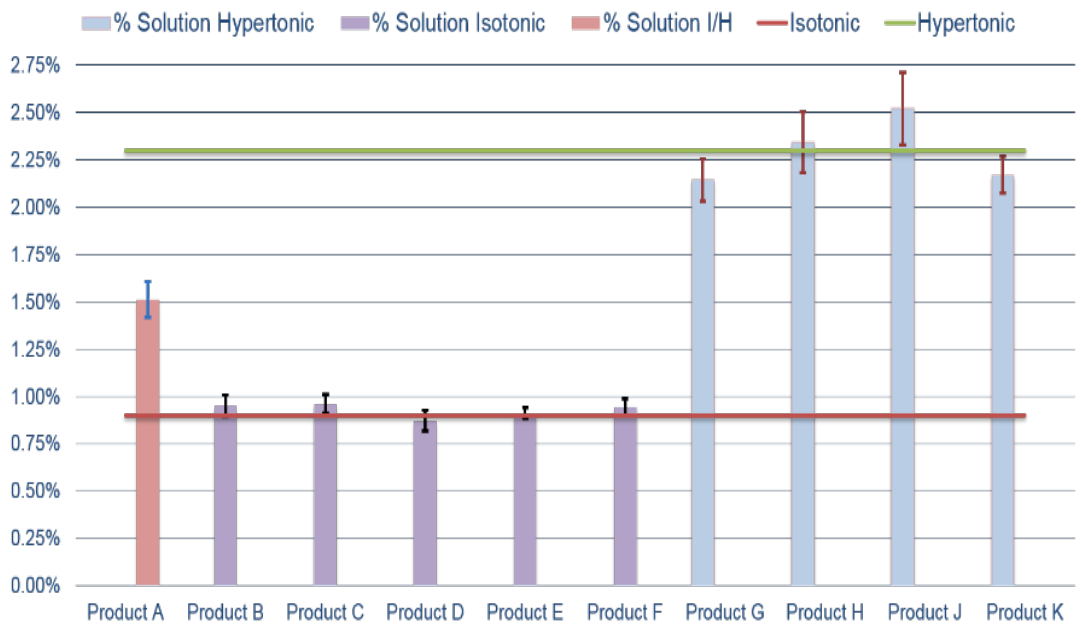


Figure 18 - % Saline solution for products A - K determined by IC – the red line indicating isotonic levels and the green line indicating the expected level for hypertonic solutions with the points indicating the mean values and the error bars indicating standard deviation for 4 replicates

These results confirm the isotonic and hypertonic nature of the products but were unable to quantify concentrations of the other lower concentration bulk cations in the products using the commercially purchased 6-ion standard. All readings for K^+, Mg^{2+} and Ca^2 fell below the smallest calibration standard as seen in Experimental section 3.8.2. Consequently, a 4 multi-ion standard, based on these concentrations, was used in future analysis to enable quantification of the data - experimental section 3.8.3.

3.1.3 **Nasal Spray 10 Product Optimised Bulk Metal Analysis.**

Sample solutions were prepared to an estimated 100 mg/L based on the estimated Na^+ content. In total, 40 samples were analysed, 10 products, 2 batches and replicates. Samples of Halen Môn salt, spa salt (unwashed), pure salt (washed), 0.1 micron filtered sea water, and fully filtered seawater, were prepared for analysis. Details can be seen in experimental section 3.8.3.

Calibration curves for Ca^{2+} (A), Mg^{2+} (B), K^+ (C) and Na^+ (D) can be seen in Appendix D.

The LOD and LOQ were calculated for Na^+, K^+, Mg^{2+} and Ca^{2+} from replicates of the blank and can be seen in Table 13. All sample concentrations were greater than the LOQ.

Table 13 - The LOD and LOQ for Na^+, K^+, Mg^{2+} and Ca^{2+} calculated from the mean cation concentration in the blank

	Mean cation oncentration in the blank (mg/L)	SD (mg/L)	LoD (mg/L)	LoQ (mg/L)
Na^+	1.71	0.15	2.48	3.25
K^+	0.01	0.004	0.03	0.05
Mg^{2+}	0.01	0.003	0.02	0.03
Ca^{2+}	0.04	0.02	0.16	0.28

The results for the commercial products showed concentrations within a similar range to previous analysis for % saline solution as seen in Figure 19.

Halen Môn (HM) salt and seawater were analysed alongside the commercial products Spa salt, pre-washed salt, and pure salt, washed salt, were selected for analysis to see if there was a difference in the bulk cation concentrations.

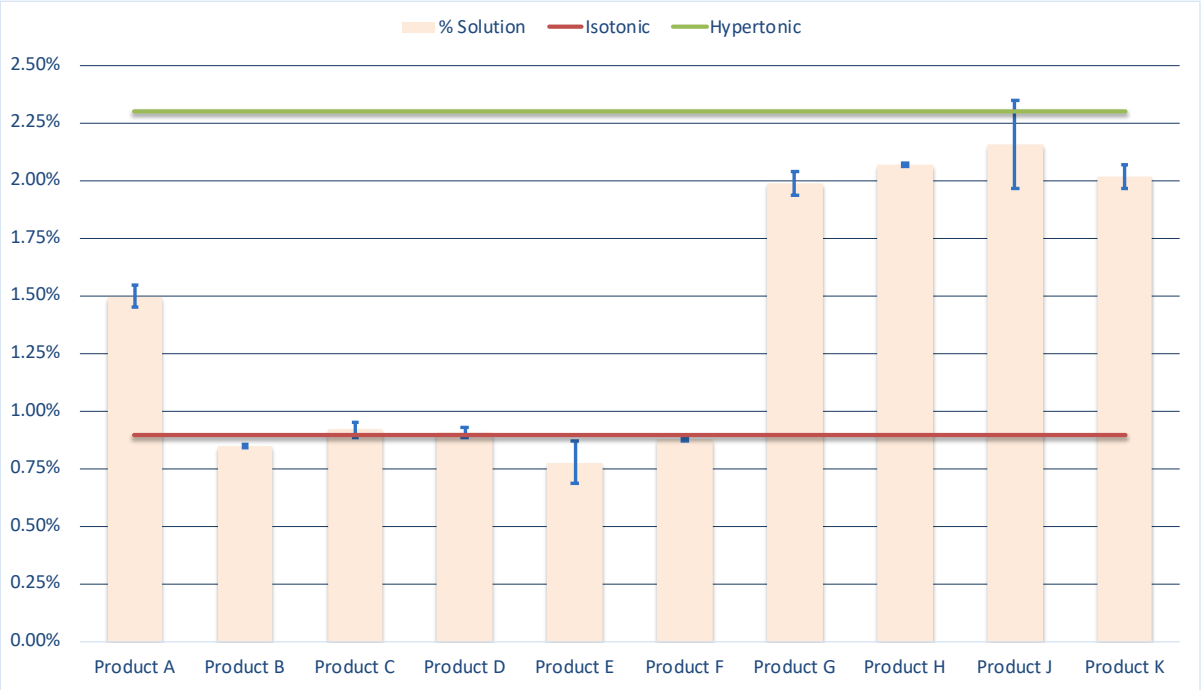


Figure 19 - % saline solution optimised bulk metal analysis for products A - K determined by IC – the red line indicating isotonic levels and the green line indicating the expected level for hypertonic solutions the points indicating the mean values and the error bars indicating standard deviation for 4 replicates

The Na^+ content in the diluted 2 g sample in mg/L is shown in **Error! Reference source not found.**. This shows there is difference in the Na^+ ions in the unwashed spa salt when compared to the washed pure food standard salt.

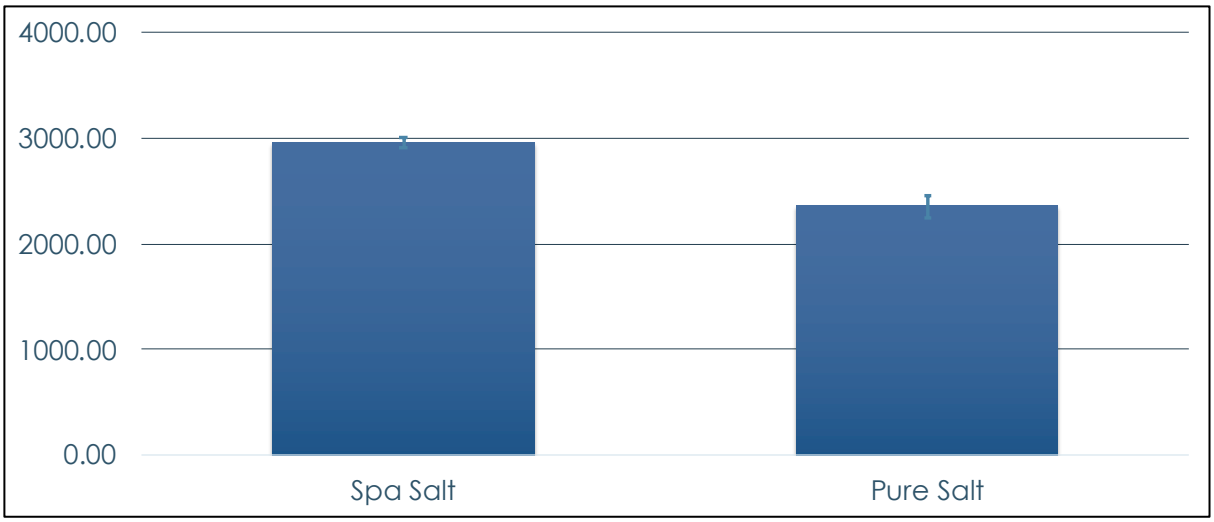


Figure 20 – Na^+ concentration in mg/L in Halen Môn spa and pure salt with the points indicating the mean values and the error bars indicating standard deviation for 4 replicates

This is lower than the concentration seen in the Himalayan salt product. This can be seen in Table 14.

Table 14 - Concentrations for Na^+ in mg/L for Himalayan salt (product D) and HM spa and pure sea salt

	Product D	SD	Spa Salt	SD	Pure Salt	SD
Na^+	3576.79	76.93	2956.38	54.33	2353.21	104.7
K^+	<0.000	<0.000	10.59	0.25	20.79	1.34
Mg^{2+}	<0.000	<0.000	22.95	6.83	58.05	1.91
Ca^{2+}	<0.000	<0.000	115.94	11.05	42.74	11.77

This could be due to the nature of sea salt. Sea salt contains K^+, Mg^{2+} and Ca^{2+} salts which all add to its salinity. To take this into account, more salt would be needed to reach a comparable Na^+ concentration.

The seawater used in the salt producing process at Halen Môn goes through a filtration process before it is used to produce its salt products. This ranges from an initial filtration using a 0.1-micron filter to being passed through a carbon filter and ultra-violet light. The Na^+ concentration in the 0.1-micron filtered seawater were within the range of normal seawater, as were K^+, Mg^{2+} and Ca^{2+} ions.[40] The Na^+ concentration is shown in mg/L in Figure 21.

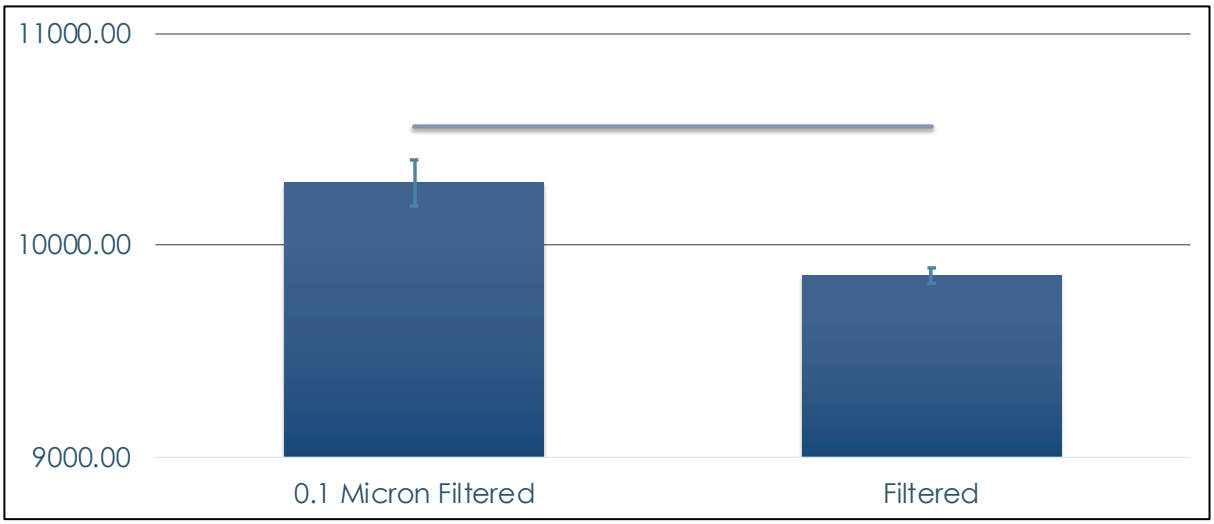


Figure 21 - Na^+ concentration in 0.1 micron filtered and fully filtered Halen Mon seawater. The points indicate the means and the error bars show standard deviation for 4 replicates with the grey bar indicating the average salinity in seawater

There was a lower Na^+ concentration in the fully filtered seawater. Table 15 shows the K^+, Mg^{2+} and Ca^{2+} concentrations in mg/L for the 10 products alongside Halen Môn salt and sea water

Table 15 - Concentrations of Na^+ in mg/L in 0.1 micron filtered and fully filter HM sea water

	0.1 Micron filtered SW	SD	Fully Filtered sea water	SD
Na^+	10293.37	112.41	9856.04	36.54
K^+	358.18	7.23	344.17	4.95
Mg^{2+}	1223.34	15.05	1175.52	26.29
Ca^{2+}	422.11	4.52	394.72	27.49

Figure 22 shows the K^+, Mg^{2+} and Ca^{2+} concentrations for the 10 products alongside Halen Môn salt and sea water. During the salt production process K^+, Mg^{2+} and Ca^{2+} ions were lost. Using 2 g of both spa and pure salt give very different concentration profiles. The spa salt, unwashed salt, has higher Na^+ and Ca^{2+} concentration than in the washed, pure salt, there is a higher K^+ and Mg^{2+} content. This can be clearly seen in the chromatogram in Figure 23.

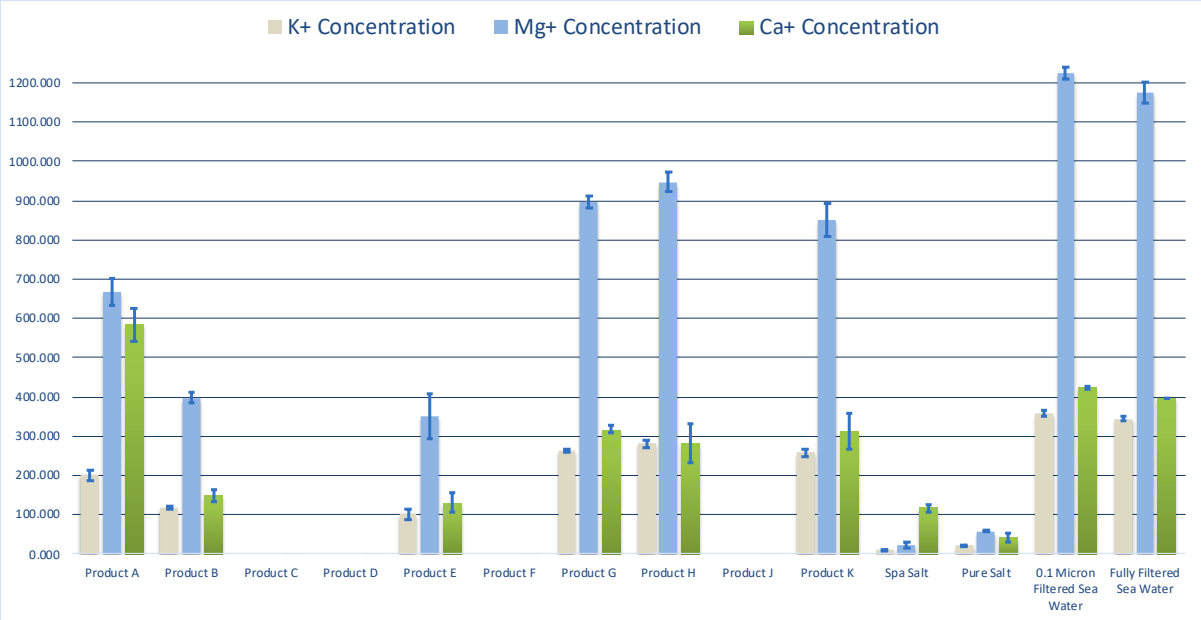


Figure 22 - K^+, Mg^{2+} and Ca^{2+} concentrations for the 10 products (A-K in the table) alongside Halen Mon salt and seawater, the error bars display standard deviation across 4 replicates and the means are represented by the points

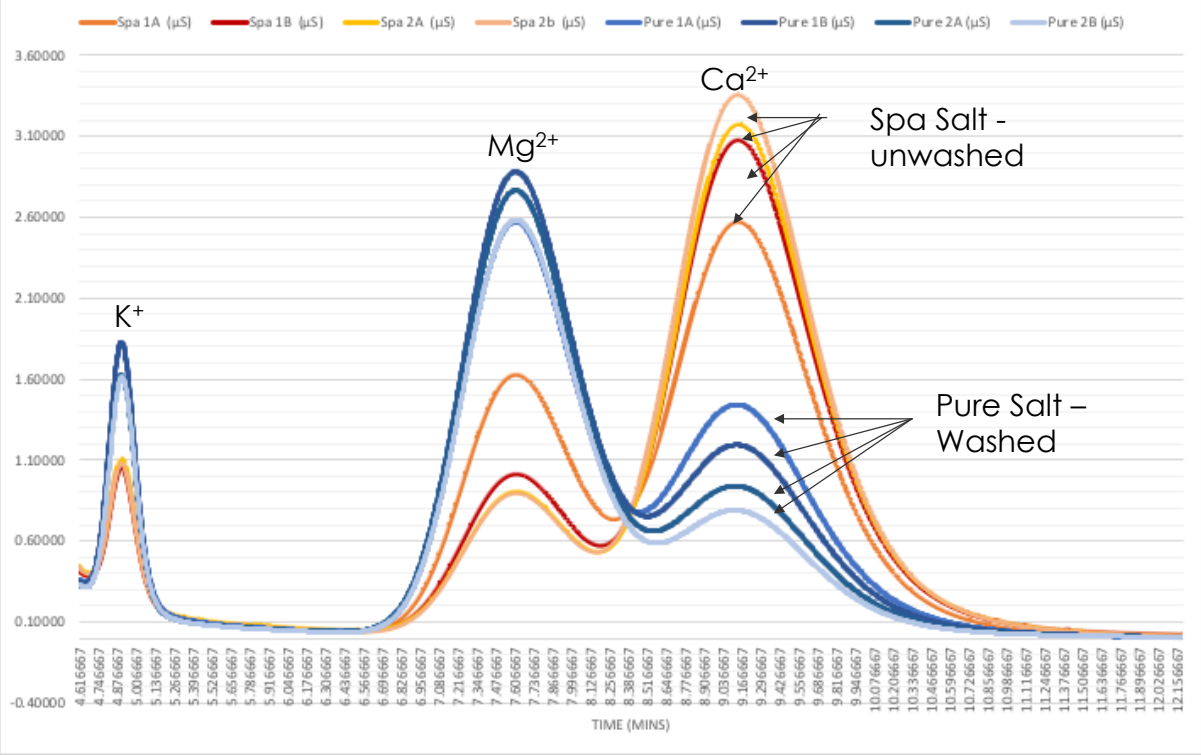


Figure 23 - Chromatogram of the ion concentration (excluding Na^+) in Halen Mon spa and pure salt

The concentrations in mg/L for products A-K with their respective standard deviations can be seen in Table 16.

Table 16 - Concentration in mg/L with SD for products A-K for the bulk metal cations

	Na^+	K^+	Mg^{2+}	Ca^{2+}
Product A	5881.71	200.04	667.58	583.16
SD	184.44	13.42	34.70	43.54
Product B	3357.84	116.60	396.44	148.41
SD	51.48	4.02	13.38	13.62
Product C	3602.93	<0.000	<0.000	<0.000
SD	100.97	<0.000	<0.000	<0.000
Product D	3576.79	<0.000	<0.000	<0.000
SD	76.93	<0.000	<0.000	<0.000
Product E	3083.36	101.50	351.42	130.96
SD	372.28	13.90	57.83	26.52
Product F	3479.22	<0.000	<0.000	<0.000
SD	48.00	<0.000	<0.000	<0.000
Product G	7835.99	262.48	895.25	317.30
SD	200.93	3.57	14.62	9.77
Product H	8147.67	280.41	947.81	283.00
SD	34.63	9.92	26.26	49.97
Product J	8482.62	<0.000	<0.000	<0.000
SD	730.95	<0.000	<0.000	<0.000
Product K	7965.47	258.27	850.55	312.59
SD	177.44	9.23	43.78	45.11

3.2 ICP-OES Analysis Nasal Products

3.2.1 ICP-OES Analysis, Full Cation Semi-qualitative Analysis.

The trace cation make-up of the 10 commercial products was unknown, therefore, analysis was performed initially in semi-quantitative mode. This has an upper detection limit of 5 mg/L and a lower limit of 10 µg/L. Dilute samples of the 10 products (concentration approx. 5 mg/L with respect to Na^+) were prepared; a total of 30 samples including replicates. Replicate HPLC water samples were also analysed. HPLC water was used in the dilution of the samples.

The ICP-OES gave statistically similar results for Na^+, K^+, Mg^{2+} and Ca^{2+} in products A - K, to IC. Figure 24 shows the comparison in the two methods with the data comparison to the results in section 3.1.2 shown in Table 17. Given the dilution factor, Cu^{2+} was not quantifiable.

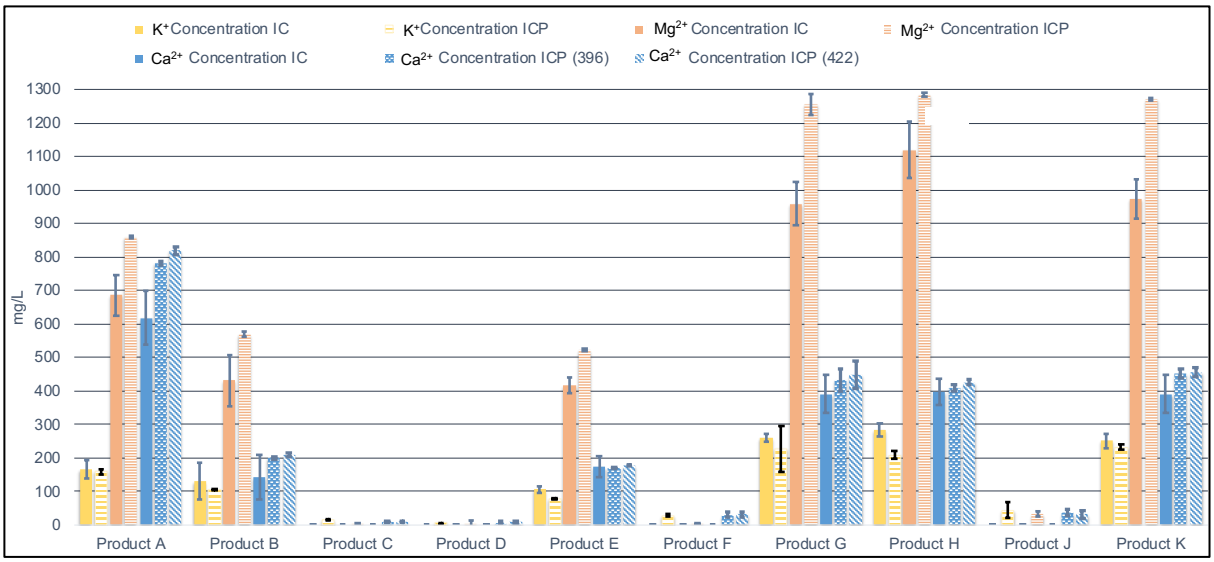


Figure 24 - Comparison of the results from IC and ICP for K^+, Mg^{2+} and Ca^2 the points indicating the mean values and the error bars indicating standard deviation for 4 replicates

Mn^{2+}, Fe^{2+} and Zn^{2+} were all present in small quantities but fell in between the LOD and the LOQ. Cu^{2+} and Mn^{2+} are listed as being enriched in Sterimar branded products, but this could not be quantified as noted above. These values can be seen in Table 18.

Table 17 – Data comparison between IC and ICP-OES concentrations in mg/L for K^+, Mg^{2+} and Ca^{2+} with SD values

	K^+ Concentration IC	Std Dev	K^+ Concentration ICP	Std Dev	Mg^{2+} Concentration IC	Std Dev	Mg^{2+} Concentration ICP	Std Dev	Ca^{2+} Concentration IC	Std Dev	Ca^{2+} Concentration ICP (396)	Std Dev
Product A	166	28	160	7	686	59	860	4	619	79	781	6
Product B	132	55	104	3	432	76	569	7	141	67	199	4
Product C	0	0	15	2	0	0	2	3	0	0	9	3
Product D	0	0	6	1	0	0	4	9	0	0	7	4
Product E	106	10	78	3	416	24	523	3	172	32	169	2
Product F	0	0	28	4	0	0	3	3	0	0	30	8
Product G	260	11	225	68	959	65	1254	31	391	56	432	34
Product H	283	20	208	11	1120	83	1284	6	397	38	409	10
Product J	0	0	45	25	0	0	33	9	0	0	35	10
Product K	250	20	232	8	973	58	1271	4	391	55	452	13

Table 18 - Concentrations of Cu^{2+}, Mn^{2+} and Fe^{2+} in Products A-K in mg/L

	Cu^{2+}	Fe^{2+}	Mn^{2+}
Product A	<0.00	0.76	0.08
Product B	<0.00	0.83	0.15
Product C	<0.00	0.37	0.04
Product D	<0.00	0.04	0.06
Product E	<0.00	1.19	0.07
Product F	<0.00	1.85	0.08
Product G	<0.00	1.12	0.32
Product H	<0.00	1.16	0.1
Product J	<0.00	4.91	0.1
Product K	<0.00	3.9	0.23

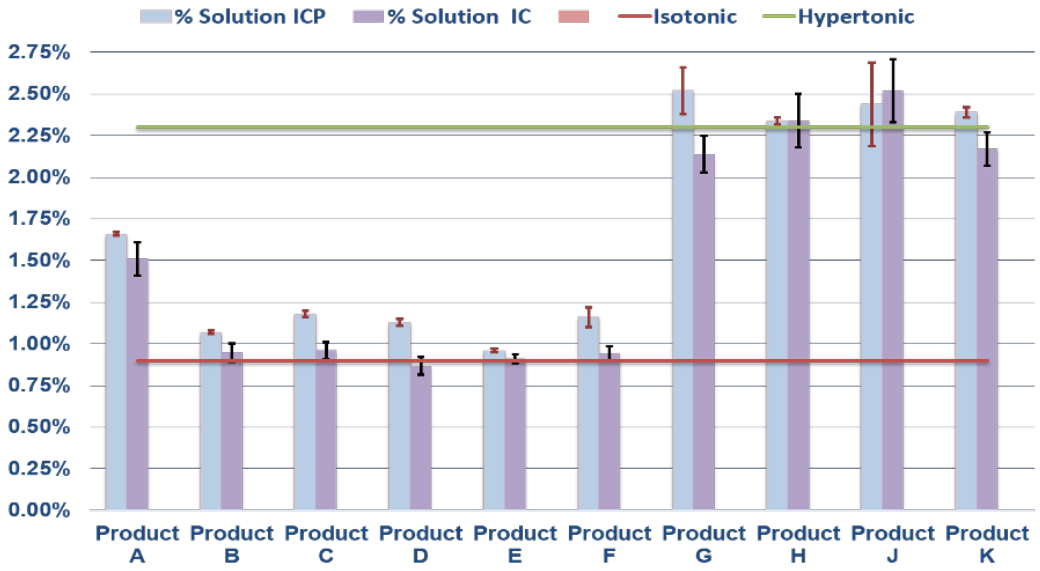


Figure 25 - Comparison between ICP and IC for % NaCl solution, the red line depicting isotonic and the green line hypertonic the points indicating the mean values and the error bars indicating standard deviation for 4 replicates

The percentage NaCl solution calculations are similar between ICP and IC. ICP results showed a greater concentration, but these results were only semi-quantitative. A comparison between the 2 methods can be seen in Figure 25. Other elements present such as sulfur and silicon[40] are consistent with minerals found in seawater.

3.2.2 **ICP-OES Analysis Full Cation Quantitative Analysis.**

From the semi-quantitative analysis carried out on the ICP-OES, and from the approximate values known for the mineral content in seawater, calibration standards

were prepared for Na^+, K^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+}. Multiple wavelengths were used to obtain calibration curves for each element. The calibration curves can be seen in Appendix E for Na^+, K^+, Mg^{2+}, and Ca^{2+}. The calibration curves for Mg^{2+} and $Ca^{2+}$and, to a lesser extent, Na^+, plateaued at higher concentrations.

The calibration curves for Cu^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+} can be seen in Appendix E

It was not possible to quantify concentrations of Fe^{2+}, Mn^{2+} and Zn^{2+}, in any of the samples because the values fell below the limit of quantification.

Product A states that it is “enriched with manganese”. The concentration of Mn^{2+} was below the limit of quantification. Products B and K are labelled as being enriched with copper. Analysis showed a Cu^{2+} concentration of approximately 0.5 mg/L in product K. An increased intensity was seen in product B, but the concentration was below the limit of quantification.

Concentrations for Na^+, K^+, Mg^{2+}, and Ca^{2+} were determined. Table 19 shows the concentrations obtained for Na^+ using ICP with a direct comparison to data obtained using IC. Figure 26 displays the comparison pictorially. Both methods give statistically similar results.

Table 19 - Na^+ concentration in the 10 commercial products & Halen Mon seawater with a comparison to IC.in mg/L

	ICP-OES	SD	IC	SD
Product A	5582.27	19.28	5881.71	184.44
Product B	3443.55	33.61	3357.84	51.48
Product C	3524.05	114.25	3602.93	100.97
Product D	3454.64	14.18	3576.79	76.93
Product E	3305.63	91.23	3083.36	372.28
Product F	3401.13	30.06	3479.22	48.00
Product G	7937.30	47.57	7835.99	200.93
Product H	8111.10	94.90	8147.67	34.63
Product J	8677.33	69.54	8482.62	730.95
Product K	7766.67	137.21	7965.47	177.44
HM SW filtered 1	10211.93	69.38	10293.37	112.41
HM SW filtered 2	10429.55	64.89	9856.04	36.54

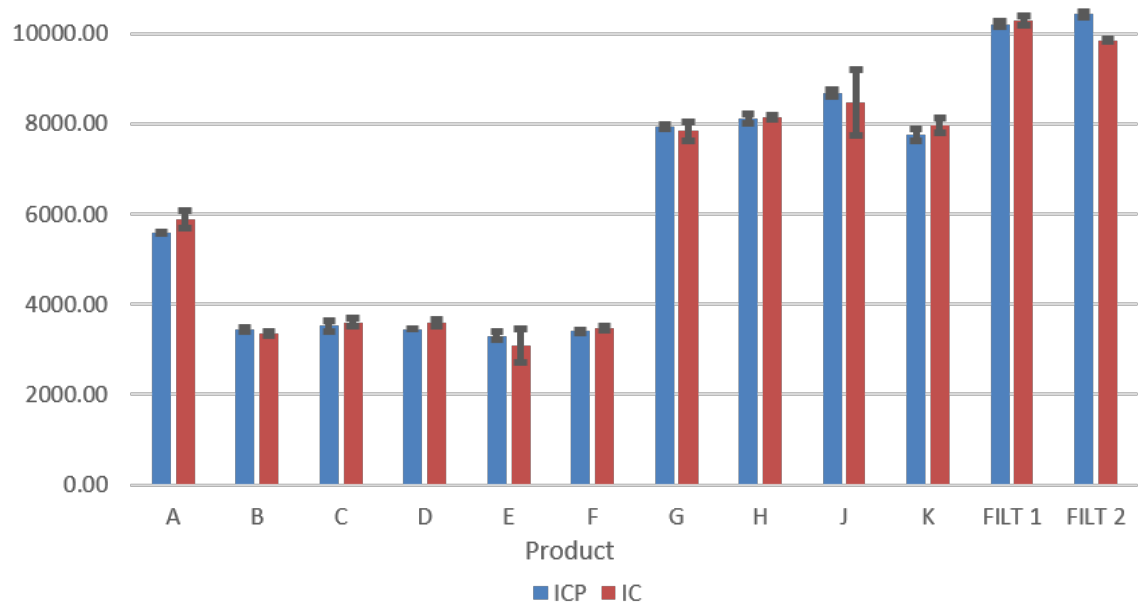


Figure 26 – Chart depicting the comparison of Na^+ concentration using ICP and IC in mg/L, the points indicating the mean values and the error bars indicating standard deviation for 4 replicates

The concentrations for K^+, Mg^{2+}, and Ca^{2+} can be seen in Table 20. with the comparable results from previous IC analysis.

Table 20 Ca^{2+}, K^+ and Mg^{2+} concentration in the 10 commercial products & Halen Mon seawater with a comparison to IC, all concentrations in mg/L

	Ca^{2+}				K^+				Mg^{2+}			
	ICP-OES	SD	IC	SD	ICP-OES	SD	IC	SD	ICP-OES	SD	IC	SD
Product A	680.34	4.83	583.16	43.54	211.39	1.44	200.04	13.42	680.04	3.01	667.58	34.70
Product B	154.68	2.35	148.41	13.62	137.29	1.05	116.60	4.02	444.21	4.91	396.44	13.38
Product C												
Product D												
Product E	145.39	4.30	130.96	26.52	128.55	4.30	101.50	13.90	426.12	11.76	351.42	57.83
Product F												
Product G	343.71	2.43	317.30	9.77	300.36	2.47	262.48	3.57	1008.17	1.72	895.25	14.62
Product H	356.61	7.32	283.00	49.97	335.06	0.51	280.41	9.92	1043.90	5.12	947.81	26.26
Product J												
Product K	335.31	7.79	312.59	45.11	297.73	7.61	258.27	9.23	949.70	20.43	850.55	43.78
HM SW filtered 1	463.94	0.73	422.11	4.52	411.29	2.07	358.18	7.23	1313.49	12.46	1223.34	15.05
HM SW filtered 2	479.01	0.67	394.72	27.49	423.15	1.56	344.17	4.95	1344.69	2.39	1175.52	26.29

Figure 27 shows this pictorially.

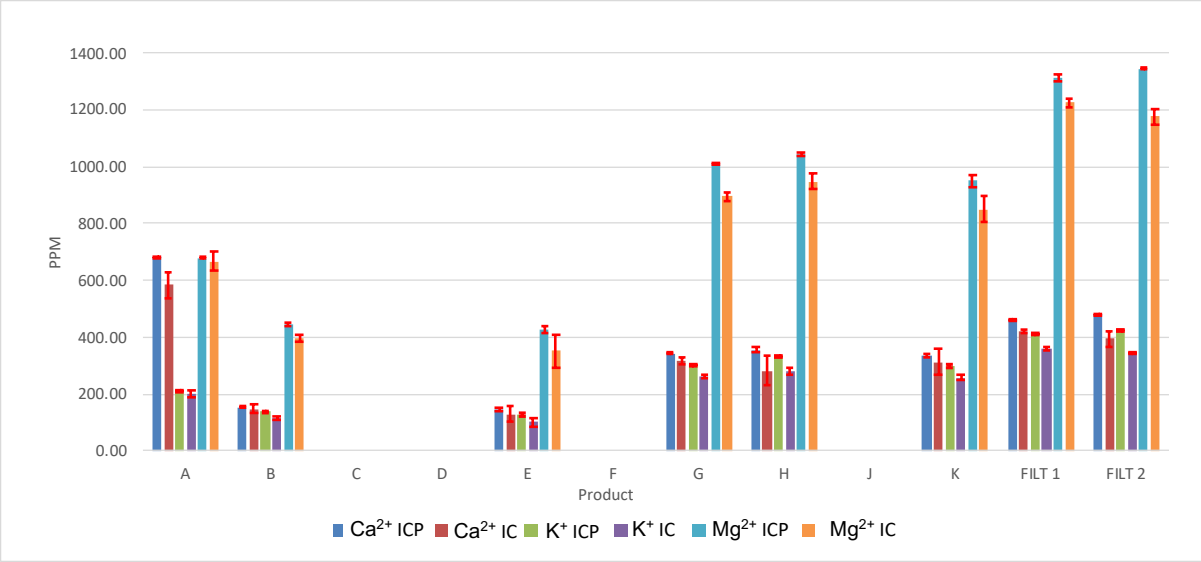


Figure 27 - Chart displaying the comparison of Ca^{2+}, K^{+} and Mg^{2+} concentration using ICP and IC, the points indicating the mean values and the error bars indicating standard deviation for 4 replicates

Products C, D, F and J are only saline solutions and do not contain any other trace metals or minerals.

Product A states it contains added Ca^{2+}. This is evident from both ICP and IC analysis. Product A is a moderately hypertonic solution, 1.5% solution, and contains four times as much Ca^{2+} as the isotonic products B and E, and almost twice as much as the hypertonic products G, H and K. The Ca^{2+} content in product A is greater than that in seawater.

The concentration ratios of the cations in each of the products is consistent in most of the products analysed. These can be seen in Table 21. The molar ratio of Na^{+} to K^{+} is approx. 44:1, Na^{+} to Mg^{2+} approx. 8:1 and Na^{+} to Ca^{2+} approx. 23:1 in all seawater products including the seawater samples. The only exception is product A, which contains additional Ca^{2+}. The Na^{+} to Ca^{2+} ratio is approx. 14:1.

Table 21 - the ratios of cations in commercial products

	A	B	C	D	E	F	G	H	J	K	FILT 1	FILT 2
Na^{+} / Ca^{2+}	14	39			40		40	40		40	38	38
Na^{+} / K^{+}	45	43			44		45	41		44	42	42
Na^{+} / Mg^{2+}	9	8			8		8	8		9	8	8

3.3 **AAS Analysis of Commercial Nasal Products**

AAS is another useful technique to measure trace metals in solutions but does not have the sensitivity of ICP.

The ppm values for trace metals in seawater (at approximately 2.7% solution) are:[40]

Na^+	10561 ppm	459.2 mmol/L
K^+	380 ppm	9.7 mmol/L
Ca^{2+}	400 ppm	9.8 mmol/L
Mg^{2+}	1232 ppm	52.3 mmol/L
Fe^{2+}	0.2 ppm	0.0004 mmol/L
Cu^{2+}	0.09 ppm	0.0014 mmol/L
Zn^{2+}	0.014 ppm	0.00002 mmol/L
Mn^{2+}	0.01 ppm	0.00019 mmol/L

For isotonic products the ppm values would be 1/3rd of these values. These values mean that some of the trace metals fall below the LOD even before the products are diluted to bring Na^+ within a working range (15 – 800 ppm). Zn^{2+} (LOD 0.01 – 2 ppm), Cu^{2+} (LOD 0.03 – 10 ppm), Fe^{2+} (LOD 0.06 - 15 ppm), and Mn^{2+} (LOD 0.02 – 5 ppm), fall below the detectable range, therefore AAS was only used to analyse for bulk metals.

3.3.1 **AAS Bulk Metal Analysis of 6 Commercial Seawater Products**

Previous analysis had concentrated on 10 commercial products. These can be seen in Table 8. These products were initially analysed via ion chromatography (IC), and inductively coupled plasma optical emission spectroscopy (ICP-OES) to get a picture of their cation composition. This analysis was carried out with a limited number of replicates. From these findings 6 products, all seawater based were taken forward for further analysis alongside sea water. are products A, B, E, G, H and K from Table 8.

Analysis was performed using 10 replicates of each product and seawater. During preparation, samples were serial diluted, using micro volumes of each product in 50 mL of ultra-pure water via micro pipette.

Na^+, K^+, Mg^{2+}, and Ca^{2+} fall within the detectable range as seen in Table 22, however, there were still challenges. Na^+ and K^+ both partly ionise in the air/acetylene flame, while Mg^{2+} and Ca^{2+} both experience interferences. Initially IC had been used to analyse for the bulk metals, however, this is very time consuming at a minimum of 30 min per sample. Initial attempts to use AAS had not been successful. Samples were diluted to bring Na^+ to approximately 100 mg/L, but this did not produce replicable results for other ions. By diluting the samples further to within 0 – 1.5 mg/L range for Na^+, this gave more reproducible results.

Table 22 - the wavelengths and detection limits for Na^+, K^+, Mg^{2+}, and Ca^{2+}

	Wavelength (nm)	Detenction range (mg/L)	Wavelength (nm)	Detenction range (mg/L)	Wavelength (nm)	Detenction range (mg/L)
Na^+	589.0	0.002 - 1.0	589.6	0.01- 2.0	330.2/3	2 - 400
K^+	766.5	0.03 - 2.0	769.9	0.1- 6	404.4	15 - 800
Mg^{2+}	285.2	0.003 - 1	202.6	0.15 - 2.0		
Ca^{2+}	422.7	0.01- 3	239.9	2 - 800		

The calibration charts for can be seen in Appendix C.

3.3.1.1 Na^+ Analysis

The products and seawater (SW) samples were diluted to approximately 0.5 mg/L to bring the products within the range of the calibration standards. The factored-up results can be seen in Table 23.

Table 23 - Na^+ concentrations in commercial products and seawater in mg/L

Na^+	A	B	E	G	H	K	SW
Mean	5978	3527	3476	9251	8740	9326	9605
Standard Error	153	80	132	296	193	336	380
Median	5899	3638	3483	9312	8695	9075	9610
Standard Deviation	485	254	416	935	612	1062	1201
Sample Variance	235598	64355	172931	875039	374083	1127052	1442351
Kurtosis	0.21	-1.37	0.33	-1.20	0.52	2.02	-0.47
Skewness	0.54	-0.55	0.22	-0.15	0.22	1.59	-0.43
Range	1640	719	1435	2747	2177	3339	3601
Minimum	5282	3093	2823	7811	7701	8358	7385
Maximum	6922	3812	4259	10558	9878	11698	10986
Sum	59778	35273	34758	92506	87399	93259	96050
Count	10	10	10	10	10	10	10
Confidence Level(95.0%)	347	181	297	669	438	759	859
Lower Bound	5631	3346	3178	8581	8302	8566	8746
Upper Bound	6325	3709	3773	9920	9177	10085	10464

The kurtosis[85] value gives an indication as to the presence of outliers in the data set. A normal distribution would have a kurtosis of 3. If the kurtosis value is above 3 the distribution would be leptokurtic. This would indicate the likelihood of greater and more extreme outliers. A kurtosis below 3 makes the distribution platykurtic. You would expect to see fewer and less extreme outliers. The kurtosis values all fall below 3 giving a platykurtic distribution. The numbers do not suggest any extreme outliers, but outlier analysis was performed to see if there were any outliers present. Product K contained 2 outliers. These can be seen in Figure 28.

The revised data removing the outliers can be seen in mg/L in Table 24. Table 25. shows the data in mmol/L and includes the % NaCl for each product. These values are within the expected range, showing product A to be mildly hypertonic approximately 1.5%, B and E isotonic approximately 0.9% and G, H and K hypertonic approximately 2.3%.

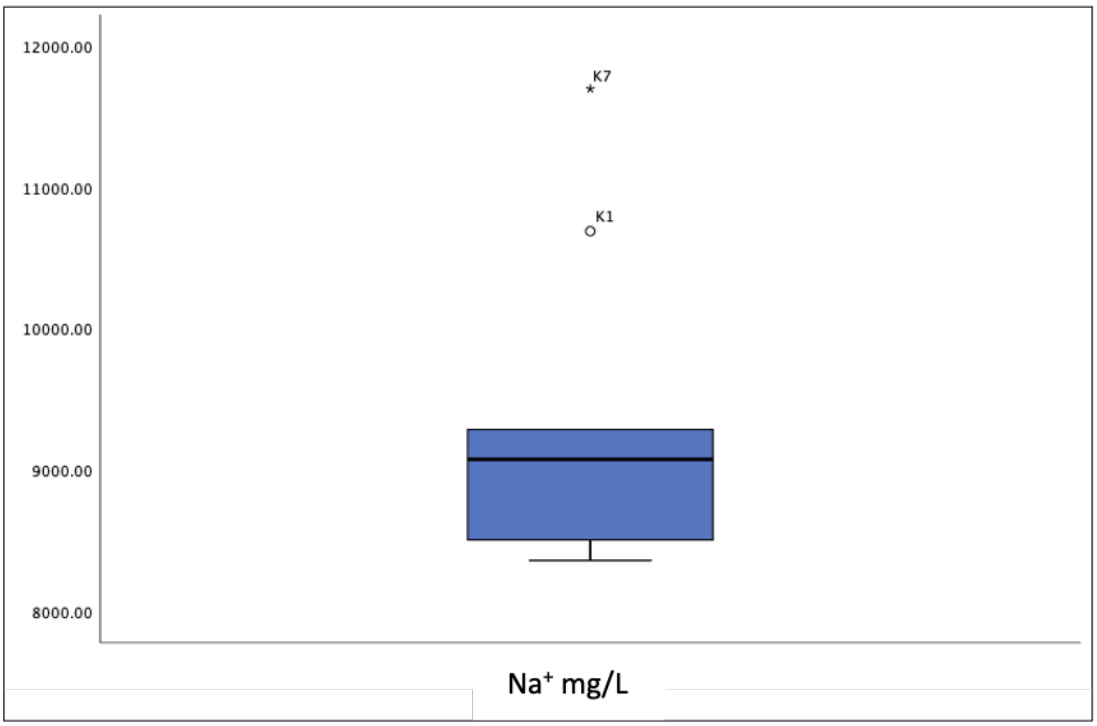


Figure 28 - Box plot from outlier analysis for Na^+ showing 2 outliers in product K

The mean for the hypertonic products was slightly higher than expected, however, it does fall within acceptable upper and lower bounds. A 0.9% solution would contain in the region of 3540 mg/L of Na^+. This fits with the values in products B and E. The seawater samples show a lower salinity than in standard sea water. Salinity varies in different areas and can vary with temperature.

A visual representation of the data can be seen in Figure 29 showing the mean with upper and lower bounds. The results show that the concentrations of Na^+ fell within the expected range for each product. The table above gives the mean, standard deviation, and the confidence level. The result gives a 95% confidence level that the Na^+ concentration of the product falls between the lower and upper bound values.

Table 24 - Na^+ concentrations in commercial products and seawater in mg/L minus outliers

Na^+	A	B	E	G	H	K	SW
Mean	5978	3527	3476	9251	8740	8859	9605
Standard Error	153	80	132	296	193	128	380
Median	5899	3638	3483	9312	8695	8983	9610
Standard Deviation	485	254	416	935	612	362	1201
Sample Variance	235598	64355	172931	875039	374083	130880	1442351
Kurtosis	0.21	-1.37	0.33	-1.20	0.52	-1.87	-0.47
Skewness	0.54	-0.55	0.22	-0.15	0.22	-0.41	-0.43
Range	1640	719	1435	2747	2177	928	3601
Minimum	5282	3093	2823	7811	7701	8358	7385
Maximum	6922	3812	4259	10558	9878	9286	10986
Sum	59778	35273	34758	92506	87399	70872	96050
Count	10	10	10	10	10	8	10
Confidence Level(95.0%)	347	181	297	669	438	302	859
Lower Bound	5631	3346	3178	8581	8302	8566	8746
Upper Bound	6325	3709	3773	9920	9177	10085	10464

Table 25 - Na^+ concentrations in commercial products and seawater in mmol/L minus outliers

Na^+	A	B	E	G	H	K	SW
Mean	260	153	151	402	380	385	418
Standard Error	7	3	6	13	8	6	17
Median	257	158	152	405	378	391	418
Standard Deviation	21	11	18	41	27	16	52
Sample Variance	446	122	327	1656	708	248	2729
Kurtosis	0.21	-1.37	0.33	-1.20	0.52	-1.87	-0.47
Skewness	0.54	-0.55	0.22	-0.15	0.22	-0.41	-0.43
Range	71	31	62	119	95	40	157
Minimum	230	135	123	340	335	364	321
Maximum	301	166	185	459	430	404	478
Sum	2600	1534	1512	4024	3802	3083	4178
Count	10	10	10	10	10	8	10
Confidence Level(95.0%)	15	8	13	29	19	13	37
Lower Bound	245	146	138	373	361	372	380
Upper Bound	275	161	164	431	399	399	455

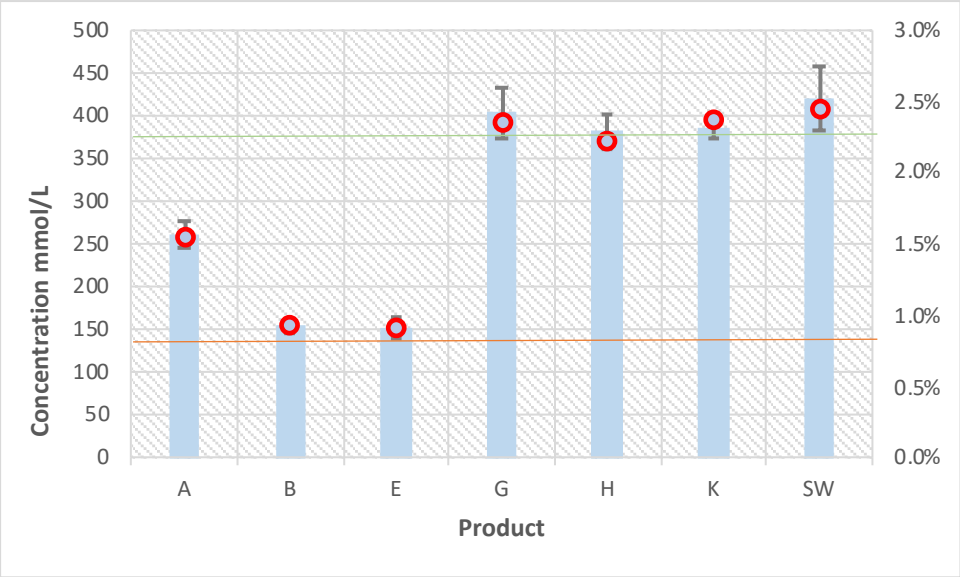


Figure 29 - Visual representation of Na^+ concentrations (mmol/L) in the 6 commercial products with the upper and lower bound limits shown by the error bars. The red circles indicate the % NaCl in each product with the orange line indicating an isotonic product and the green line a hypertonic solution.

This confirms that the products fall within the anticipated % solution ranges for Na^+ when considering the mean +/- standard deviation.

3.3.1.2 K^+ Analysis

The six nasal spray samples and the seawater samples were diluted to between 1.0 and 1.5 ppm to bring the concentration within the calibration range. The factored-up results in mmol/L can be seen in Table 26.

The kurtosis values are all below 3, giving a platykurtic distribution. There is unlikely to be many outliers. The only probability of an outlier is product H. Outlier analysis was performed on the dataset using SPSS. The outlier seen was in product H and can be seen in Figure 30.

Table 26 - AAS results for K^+. All concentrations are in mg/L

K^+	A	B	E	G	H	K	SW
Mean	225	136	137	312	333	348	417
Standard Error	2	1	1	3	2	4	3
Median	225	136	137	313	332	349	415
Standard Deviation	5	4	3	9	8	11	10
Sample Variance	23	16	9	89	62	131	108
Kurtosis	-0.47	0.38	-0.08	-0.82	2.01	-0.25	-1.12
Skewness	0.16	-0.10	-0.17	-0.40	0.93	0.01	0.15
Range	15	14	10	27	28	38	31
Minimum	219	128	132	298	322	330	401
Maximum	233	143	142	325	350	368	431
Sum	2254	1356	1371	3124	3327	3483	4167
Count	10	10	10	10	10	10	10
Confidence Level(95.0%)	3	3	2	7	6	8	7
Lower Bound	222	133	135	306	327	340	409
Upper Bound	229	139	139	319	338	356	424

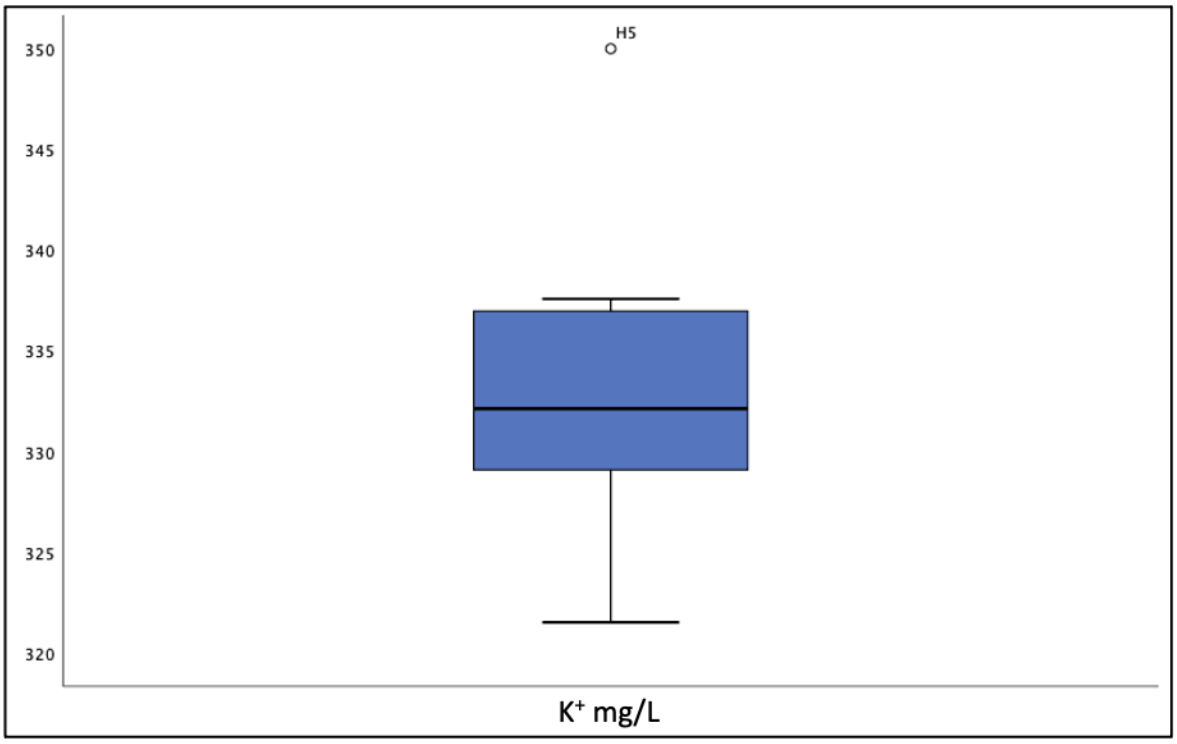


Figure 30 – Box plot from outlier analysis for K^+ showing 1 outlier in product H

The results for K^+ minus the outlier can be seen in Table 27 in mg/L and in Table 28 in mmol/L.

Table 27 - AAS results for K^+. All concentrations are in mg/L minus outlier

K^+	*A*	*B*	*E*	*G*	*H*	*K*	*SW*
Mean	225	136	137	312	331	348	417
Standard Error	2	1	1	3	2	4	3
Median	225	136	137	313	332	349	415
Standard Deviation	5	4	3	9	5	11	10
Sample Variance	23	16	9	89	28	131	108
Kurtosis	-0.47	0.38	-0.08	-0.82	-0.20	-0.25	-1.12
Skewness	0.16	-0.10	-0.17	-0.40	-0.57	0.01	0.15
Range	15	14	10	27	16	38	31
Minimum	219	128	132	298	322	330	401
Maximum	233	143	142	325	338	368	431
Sum	2254	1356	1371	3124	2977	3483	4167
Count	10	10	10	10	9	10	10
Confidence Level(95.0%)	3	3	2	7	4	8	7
Lower Bound	222	133	135	306	327	340	409
Upper Bound	229	139	139	319	335	356	424

Table 28 - AAS results for K^+. All concentrations are in mmol/L minus outlier

K^+	*A*	*B*	*E*	*G*	*H*	*K*	*SW*
Mean	5.8	3.5	3.5	8.0	8.5	8.9	10.7
Standard Error	0.0	0.0	0.0	0.1	0.0	0.1	0.1
Median	5.8	3.5	3.5	8.0	8.5	8.9	10.6
Standard Deviation	0.1	0.1	0.1	0.2	0.1	0.3	0.3
Sample Variance	0.0	0.0	0.0	0.1	0.0	0.1	0.1
Kurtosis	-0.47	0.38	-0.07	-0.82	-0.20	-0.25	-1.12
Skewness	0.16	-0.10	-0.18	-0.40	-0.57	0.01	0.15
Range	0.4	0.4	0.3	0.7	0.4	1.0	0.8
Minimum	5.6	3.3	3.4	7.6	8.2	8.4	10.2
Maximum	6.0	3.6	3.6	8.3	8.6	9.4	11.0
Sum	57.6	34.7	35.1	79.9	76.1	89.1	106.6
Count	10.0	10.0	10.0	10.0	9.0	10.0	10.0
Confidence Level(95.0%)	0.1	0.1	0.1	0.2	0.1	0.2	0.2
Lower Bound	5.7	3.4	3.5	7.8	8.4	8.7	10.5
Upper Bound	5.9	3.5	3.6	8.2	8.6	9.1	10.8

A visual representation of this data can be seen in Figure 31.

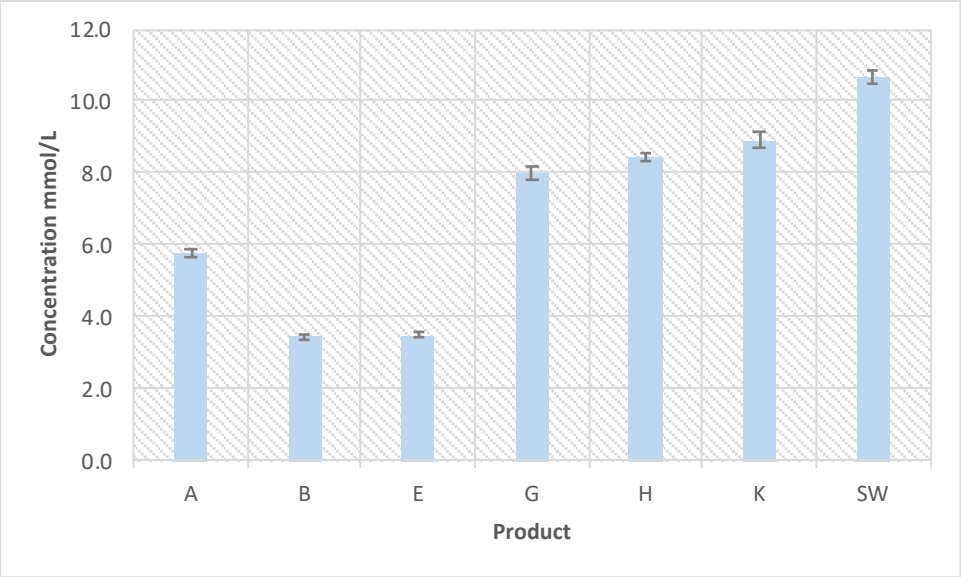


Figure 31 - Visual representation of K^+ concentrations (mmol/L) in the 6 commercial products with the upper and lower bound limits marked with error bars

In general seawater has an approximate concentration of 308 mg/L (9.7 mmol/L), based on a salinity of 2.7% solution.[70] This may vary by location, but it gives a representative view. The seawater in the Menai Straits (53°10'50"N 4°14'00"W) gives readings slightly higher for K^+, but slightly lower for Na^+. The 6 products fall within expected ranges for K^+ content in seawater and give consistent results.

3.3.1.3 Mg^{2+} Analysis

The six nasal spray samples and the seawater samples were diluted to approximately to 0.5 ppm to bring the concentration within the calibration range. The factored-up results in mg/L can be seen in Table 29. The kurtosis values indicate a platykurtic distribution for all products with the exception of E, E has a value of 8, indicating a leptokurtic distribution. There is a likelihood of many or extreme outliers.

Table 29 - AAS results for Mg^{2+}. All concentrations are in mg//L

Mg^{2+}	A	B	E	G	H	K	SW
Mean	577	355	324	825	878	890	1107
Standard Error	4	3	13	7	10	9	7
Median	577	355	334	825	882	891	1116
Standard Deviation	12	8	42	21	31	30	22
Sample Variance	136	71	1759	459	967	892	503
Kurtosis	-1.10	-0.09	8.56	-0.08	-1.20	-1.14	-1.00
Skewness	0.41	0.20	-2.85	-0.49	-0.35	-0.30	-0.71
Range	33	28	145	68	88	85	65
Minimum	565	341	207	785	829	845	1067
Maximum	598	370	353	853	917	930	1131
Sum	5772	3546	3235	8247	8777	8903	11066
Count	10	10	10	10	10	10	10
Confidence Level(95.0%)	8	6	30	15	22	21	16
Lower Bound	569	349	294	809	855	869	1091
Upper Bound	585	361	354	840	900	912	1123

Outlier analysis was performed on the dataset and 1 product contained a single outlier. This was in product E. This can be seen in the box plot in Figure 32.

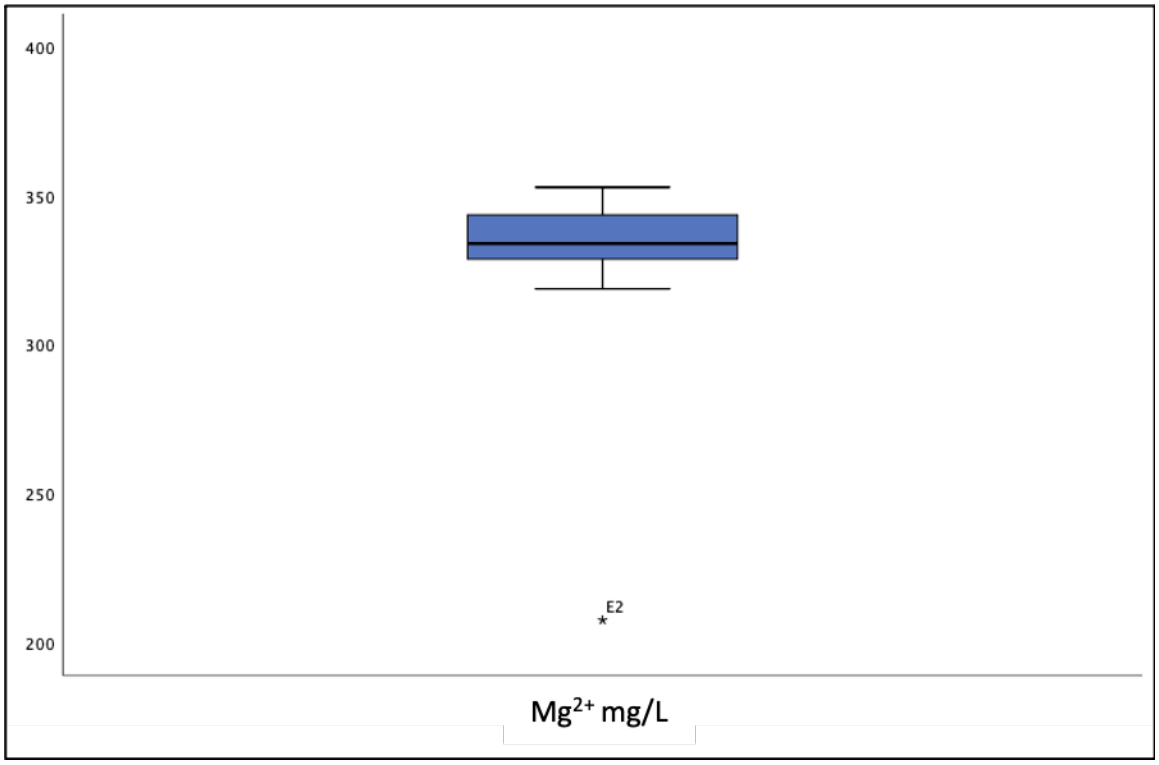


Figure 32 - Box plot from outlier analysis for Mg^{2+} showing 1 outlier in product E

The results minis the outlier can be seen in Table 30 in mg/L and in Table 31 in mmol/L. This can be seen visually in Figure 33.

Table 30 - AAS results for Mg^{2+} minus outlier. All concentrations are in mg//L

Mg^{2+}	A	B	E	G	H	K	SW
Mean	577	355	336	825	878	890	1107
Standard Error	4	3	3	7	10	9	7
Median	577	355	336	825	882	891	1116
Standard Deviation	12	8	10	21	31	30	22
Sample Variance	136	71	107	459	967	892	503
Kurtosis	-1.10	-0.09	-0.09	-0.08	-1.20	-1.14	-1.00
Skewness	0.41	0.20	-0.02	-0.49	-0.35	-0.30	-0.71
Range	33	28	34	68	88	85	65
Minimum	565	341	319	785	829	845	1067
Maximum	598	370	353	853	917	930	1131
Sum	5772	3546	3028	8247	8777	8903	11066
Count	10	10	9	10	10	10	10
Confidence Level(95.0%)	8	6	8	15	22	21	16
Lower Bound	569	349	328	809	855	869	1091
Upper Bound	585	361	344	840	900	912	1123

Table 31 - AAS results for Mg^{2+} minus outlier. All concentrations are in mmol/L

Mg^{2+}	A	B	E	G	H	K	SW
Mean	23.7	14.6	13.8	33.9	36.1	36.6	45.5
Standard Error	0.2	0.1	0.1	0.3	0.4	0.4	0.3
Median	23.7	14.6	13.8	33.9	36.3	36.6	45.9
Standard Deviation	0.5	0.3	0.4	0.9	1.3	1.2	0.9
Sample Variance	0.2	0.1	0.2	0.8	1.6	1.5	0.9
Kurtosis	-1.10	-0.09	-0.09	-0.08	-1.20	-1.14	-1.00
Skewness	0.41	0.20	-0.02	-0.49	-0.35	-0.30	-0.71
Range	1.4	1.2	1.4	2.8	3.6	3.5	2.7
Minimum	23.2	14.0	13.1	32.3	34.1	34.8	43.9
Maximum	24.6	15.2	14.5	35.1	37.7	38.3	46.6
Sum	237.5	145.9	124.6	339.3	361.1	366.3	455.3
Count	10.0	10.0	9.0	10.0	10.0	10.0	10.0
Confidence Level(95.0%)	0.3	0.2	0.3	0.6	0.9	0.9	0.7
Lower Bound	23.4	14.3	13.5	33.3	35.2	35.8	44.9
Upper Bound	24.1	14.8	14.2	34.6	37.0	37.5	46.2

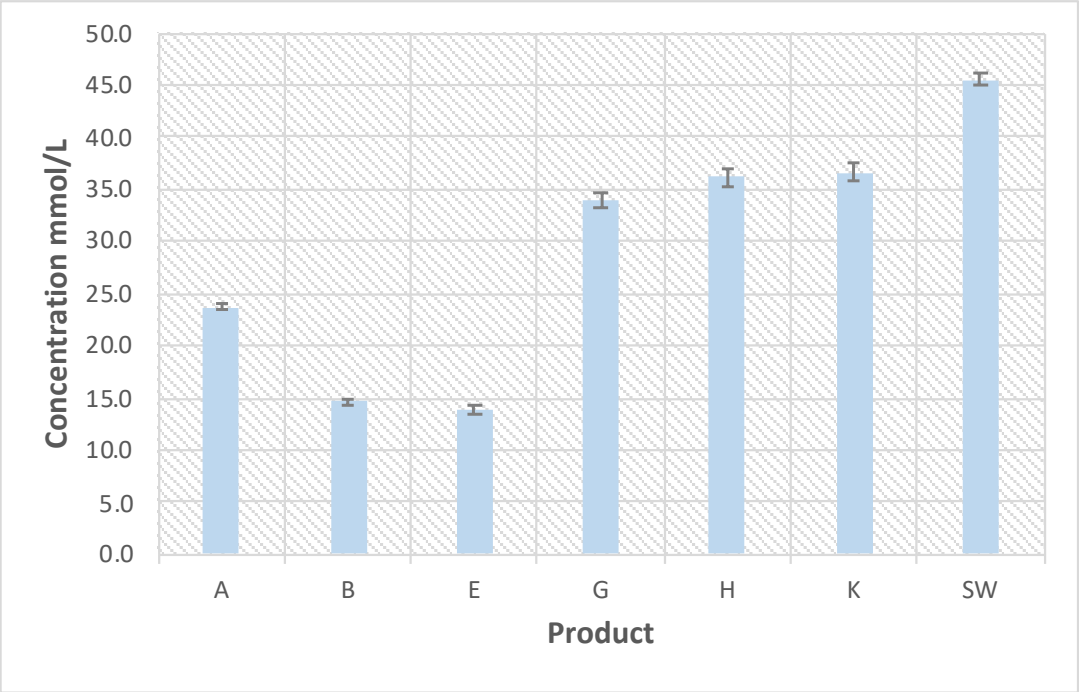


Figure 33 - Visual representation of Mg^{2+} concentrations (mmol/L) in commercial products with the upper and lower bound limits shown by error bars

In general seawater has an approximate concentration of 1232 mg/L (52 mmol/L), based on a salinity of 2.7% solution. This will vary by location, time of year and depth from which the sample is taken but gives an indicative value of concentration. The concentration of Mg^{2+} in the products is in the expected range for diluted seawater.

3.3.1.4 Ca^{2+} Analysis

The six nasal spray samples and the seawater samples were diluted to approximately to 1.0 ppm to bring the concentration within the calibration range. The factored-up results in mg/L can be seen in Table 32. The kurtosis values indicate that there are likely to me no outliers in the data. Outlier analysis was performed in SPSS and confirmed there were no outlying data points in the data set. The factored-up results in mmol/L can also be seen in Table 33.

Table 32 - AAS results for Ca^{2+}. All concentrations are in mg//L

Ca^{2+}	A	B	E	G	H	K	SW
Mean	724	182	158	354	358	345	411
Standard Error	3	1	1	1	3	5	3
Median	723	181	158	354	360	341	411
Standard Deviation	9	3	5	3	9	16	9
Sample Variance	76	12	21	10	85	259	73
Kurtosis	-0.50	-0.80	-1.54	-0.36	-1.05	-0.72	0.64
Skewness	-0.12	-0.06	-0.09	-0.23	-0.41	0.52	-0.48
Range	27	11	13	10	27	49	30
Minimum	709	176	151	348	344	325	394
Maximum	736	187	165	358	371	374	424
Sum	7235	1817	1579	3540	3583	3454	4114
Count	10	10	10	10	10	10	10
Confidence Level(95.0%)	6	2	3	2	7	12	6
Lower Bound	717	179	155	352	352	334	405
Upper Bound	730	184	161	356	365	357	417

Table 33 - AAS results for Ca^{2+}. All concentrations are in mmol//L

Ca^{2+}	A	B	E	G	H	K	SW
Mean	18.1	4.5	3.9	8.8	8.9	8.6	10.3
Standard Error	0.1	0.0	0.0	0.0	0.1	0.1	0.1
Median	18.0	4.5	4.0	8.8	9.0	8.5	10.3
Standard Deviation	0.2	0.1	0.1	0.1	0.2	0.4	0.2
Sample Variance	0.0	0.0	0.0	0.0	0.1	0.2	0.0
Kurtosis	-0.50	-0.80	-1.54	-0.36	-1.05	-0.72	0.64
Skewness	-0.12	-0.06	-0.09	-0.23	-0.41	0.52	-0.48
Range	0.7	0.3	0.3	0.3	0.7	1.2	0.7
Minimum	17.7	4.4	3.8	8.7	8.6	8.1	9.8
Maximum	18.4	4.7	4.1	8.9	9.3	9.3	10.6
Sum	180.5	45.3	39.4	88.3	89.4	86.2	102.6
Count	10	10	10	10	10	10	10
Confidence Level(95.0%)	0.2	0.1	0.1	0.1	0.2	0.3	0.2
Lower Bound	17.9	4.5	3.9	8.8	8.8	8.3	10.1
Upper Bound	18.2	4.6	4.0	8.9	9.1	8.9	10.4

This can be seen visually in Figure 34. Ca^{2+} concentration in standard seawater is approximately 400 mg/L, 9.8 mmol/L.

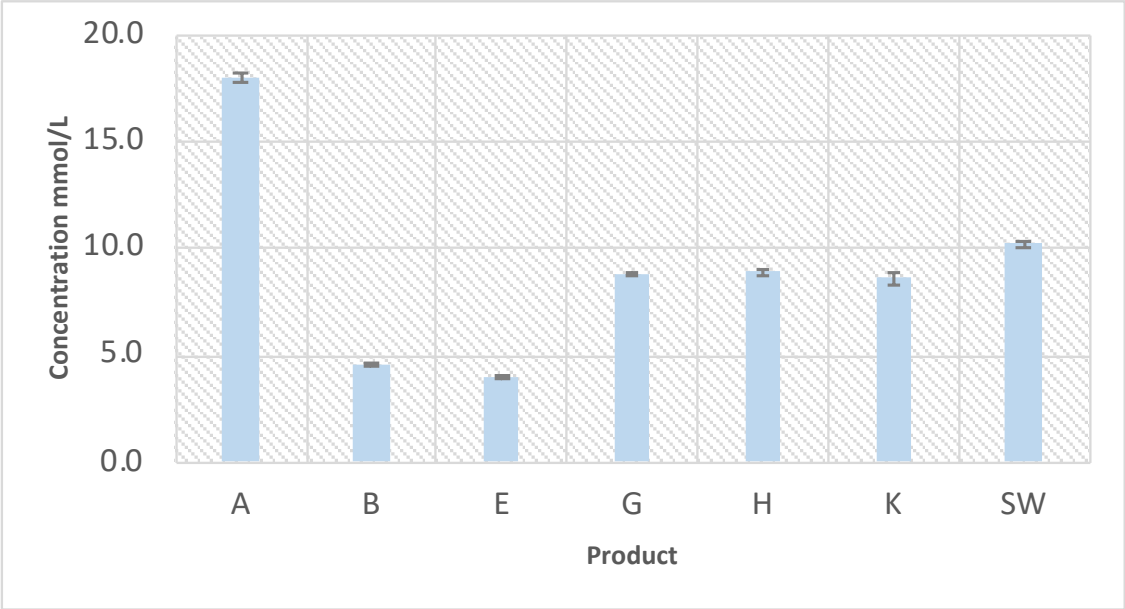


Figure 34 - Visual representation of Ca^{2+}concentrations (mmol/L) in commercial products with the upper and lower bound limits as error bars

Product A states that there is added Ca^{2+} within the product. Product A has a % solution of 1.5%, approximately half that of seawater but contains almost four times the Ca^{2+} of seawater. This shows a considerable amount of added Ca^{2+} is in the product.

3.4 Osmolality Analysis of Commercial Nasal Products

Osmolality in serum falls between 275-299 mOsm/kg. Isotonic solutions should be close to or within this range. Osmolality analysis was carried out on the 10 products, from 2 batches of product using an Advanced Systems Osmometer 3320 freezing point osmometer in the clinical chemistry laboratory at Ysbyty Gwynedd in Bangor. The results are shown in Table 34.

Figure 35 shows the relationship between osmolality and salinity (based on IC results 2) for the products. The blue bars indicate the osmolality of each product. The black markers show the salinity levels. Both measurements follow a similar trend.

Table 34 - Osmolality results for 10 commercial products with % Salinity solution from IC data

Product	Sample	Hypertonic/Isotonic	Osmolality (mOsm/kg) 1	Osmolality (mOsm/kg) 2		Mean (mOsm/kg)	Std Dev	% Nacl Solution	Std Dev
Sterimar Stop & Protect Allergy Respose	A 1	I	558	561	Product A	555.8	4.6	1.51%	0.10%
	A 2		553	551					
Sterimar Cold Defence	B 1	I	334	332	Product B	334.8	2.2	0.95%	0.05%
	B 2		336	337					
NeilMed Sinus Rinse	C 1	I	294	293	Product C	300.0	7.5	0.96%	0.05%
	C 2		307	306					
Himalyan Salt	D 1	I	273	272	Product D	281.0	9.8	0.87%	0.05%
	D 2		290	289					
Sterimar Isotonic Nasal Hygiene Spray	E 1	I	308	310	Product E	309.3	1.0	0.91%	0.03%
	E 2		310	309					
NeilMed NasaMist	F 1	I	295	298	Product F	290.8	6.8	0.95%	0.04%
	F 2		284	286					
Sinomarin Hypertonic	G 1	H	728	726	Product G	739.3	14.2	2.14%	0.11%
	G 2		751	752					
Sterimar Congestion Relief	H 1	H	776	778	Product H	775.5	2.1	2.34%	0.16%
	H 2		773	775					
NeilMed NasaMist Extra	J 1	H	708	710	Product J	729.0	23.1	2.52%	0.19%
	J 2		750	748					
Sterimar Stop & Protect Cold & Sinus Relief	K 1	H	772	770	Product K	772.0	1.6	2.17%	0.10%
	K 2		774	772					

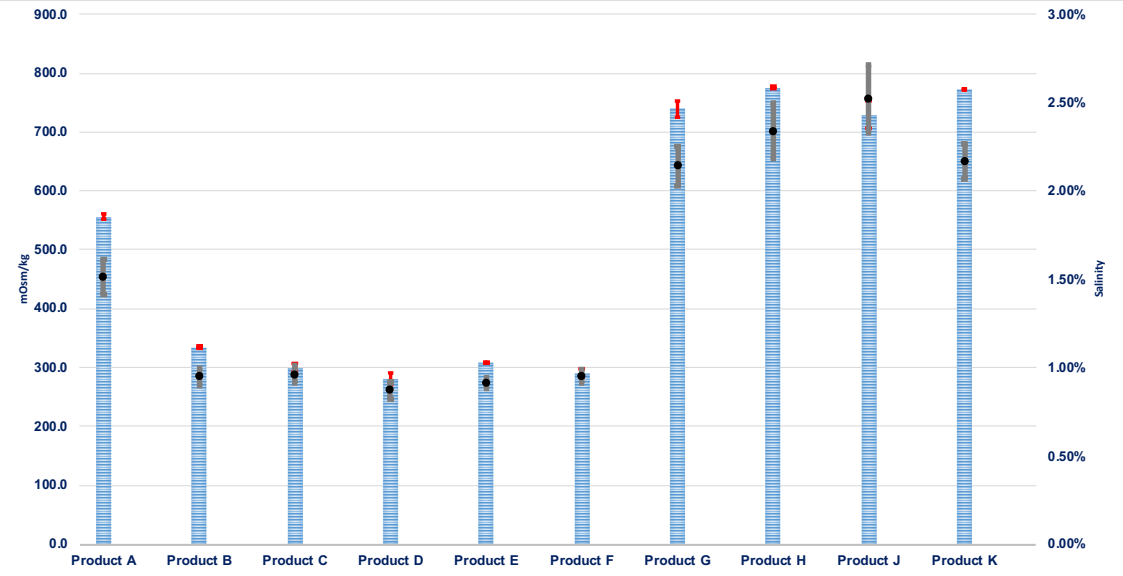


Figure 35 - Relationship between osmolality and salinity of the products. Blue bars indicating osmolality in mOsm/kg and the black dots the salinity % Solution. SD indicated by error bars.

3.5 **pH Testing of Commercial Nasal Products**

The pH of commercially purchased saline is acidic with a pH in the region of 5.5,[71] whereas sea water has a pH in the range 8.08 – 8.33.[72] The pH of blood serum is in the range 7.35 – 7.45. Commercial isotonic products need to work in harmony with the body, so the pH would need to be in a similar range. A total of 10 products, 2 batches, 5 replicates, were analysed using a Jenway 3510 pH meter. The results are provided in Table 35.

Table 35 - pH of the 10 commercial products

Product		Hypertonic /Isotonic	1	2	3	4	5	Mean	Std Dev	Product Mean	Product SD
Sterimar Stop & Protect Allergy Respose	A 1	I/H	7.30	7.32	7.35	7.32	7.36	7.33	0.02	7.34	0.03
	A 2		7.31	7.37	7.34	7.32	7.37	7.34	0.03		
Sterimar Cold Defence	B 1	I	7.09	7.00	6.99	7.00	6.99	7.01	0.04	7.01	0.04
	B 2		7.00	7.01	7.07	6.99	7.00	7.01	0.03		
NeilMed Sinus Rinse	C 1	I	7.45	7.40	7.37	7.36	7.35	7.39	0.04	7.40	0.04
	C 2		7.43	7.45	7.39	7.41	7.37	7.41	0.03		
Himalyan Salt	D 1	I	7.43	7.37	7.41	7.38	7.35	7.39	0.03	7.40	0.03
	D 2		7.38	7.41	7.43	7.39	7.41	7.40	0.02		
Sterimar Isotonic Nasal Hygiene Spray	E 1	I	7.00	6.99	6.94	6.95	6.96	6.97	0.03	6.97	0.02
	E 2		6.99	6.95	7.00	6.98	6.97	6.98	0.02		
NeilMed NasaMist	F 1	I	7.52	7.60	7.67	7.57	7.67	7.61	0.07	7.61	0.05
	F 2		7.61	7.58	7.65	7.59	7.63	7.61	0.03		
Sinomarin Hypertonic	G 1	H	7.43	7.51	7.28	7.26	7.26	7.35	0.12	7.36	0.10
	G 2		7.42	7.49	7.35	7.32	7.28	7.37	0.08		
Sterimar Congestion Relief	H 1	H	7.78	7.65	7.64	7.54	7.51	7.62	0.11	7.65	0.08
	H 2		7.69	7.65	7.68	7.58	7.74	7.67	0.06		
NeilMed NasaMist Extra	J 1	H	7.97	7.98	7.96	7.98	7.98	7.97	0.01	7.97	0.01
	J 2		7.96	7.98	7.98	7.97	7.95	7.97	0.01		
Sterimar Stop & Protect Cold & Sinus Relief	K 1	H	7.58	7.65	7.67	7.67	7.66	7.65	0.04	7.65	0.03
	K 2		7.60	7.64	7.68	7.67	7.65	7.65	0.03		

These results can also be seen in Figure 36. The pH of the products is compared to the pH values for, seawater and blood serum.[73] The isotonic solutions are all either pH neutral or fall within the pH range for blood serum, with the exception of the NeilMed Nasamist (non-seawater). The seawater isotonic products state that they are buffered solutions and pH neutral. Both NeilMed Nasamist products have a higher pH, making them more alkaline than other products in their category. Having a more alkaline solution can be more advantageous according to a review paper by Bastier *et al*.[35] Their research indicates that the pH of seawater may be the optimal pH for a nasal spray or douche.

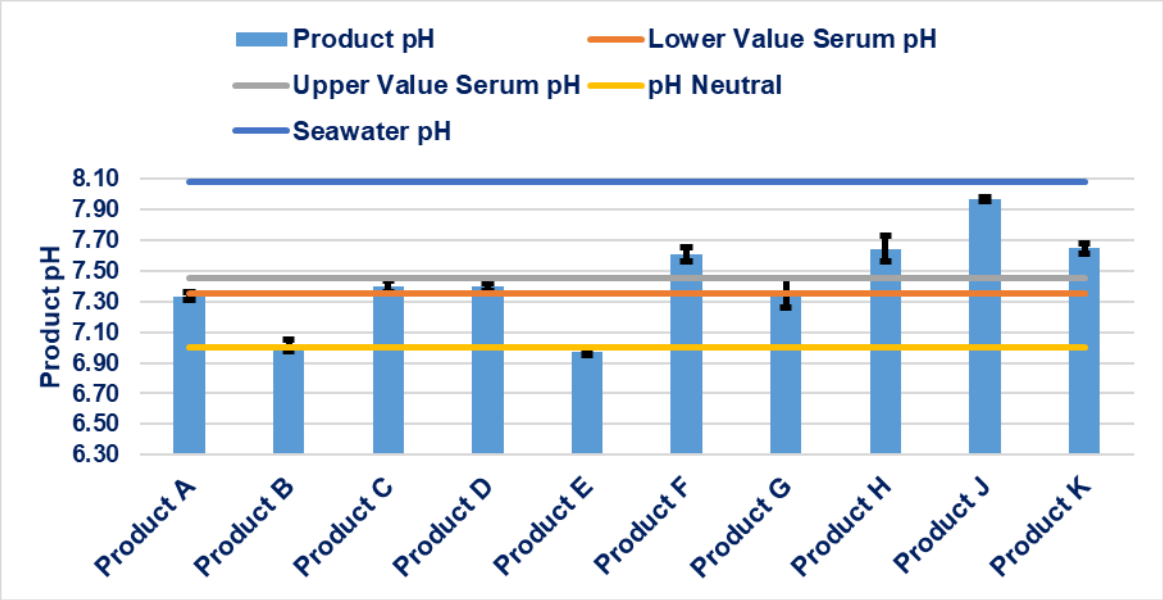


Figure 36 - pH values for 10 commercial products with a comparison to blood serum and seawater

3.6 ICP-MS Analysis of Seawater Based Nasal Products

ICP-OES was unable to quantify concentrations for the trace metal elements in seawater products as they fell below the limit of detection for the machine as seen in section 3.2.2. ICP-MS, however, can detect trace metals in the ppt (ng/mL) range and can analyse for over 50 elements simultaneously. As such, analysis with ICP-MS was undertaken.

These samples were submitted for analysis at Aberystwyth University on an Agilent 7700 ICP-MS within the Department of Geography and Earth Sciences.

3.6.1 Initial Scoping Study of Trace Metal Content of Nasal Products using ICP-MS Analysis.

The ICP-MS results gave an indication as to whether quantification of the trace metals could be made using this technique. The products needed to be diluted to approximately 1000 mg/L with respect to Na^+ to remove interferences from the high concentrations of Na^+ in the solutions. Large concentrations of Na^+ cause large deposits to build up on the cone, reducing the sensitivity.[74]

Calibration curves for Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+}, can be seen in Appendix G and the LOD and LOQ can be seen in Table 36. All concentrations were above the LOQ.

Table 36 - LOD and LOQ for Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+}

	LoD ng/mL	LoQ ng/mL
Cu^{2+}	0.102	0.647
Fe^{3+}	0.236	11.85
Mn^{2+}	0.046	0.343
Zn^{2+}	0.073	1.477

The 6 seawater-based products, which had been analysed previously, were selected for trace metal analysis to see if the addition of Ca^{2+}, Cu^{2+} and Mn^{2+} could be quantified. These products are shown in Table 37, the other 4 products were purely saline based.

Table 37 - The seawater samples selected for further analysis by ICP-MS

Product ID	**Nasal Product**
Product A	Sterimar Stop and Protect Allergy Response
Product B	Sterimar Cold Defence
Product E	Sterimar isotonic Nasal Hygiene Spray
Product G	Sinmarin Hypertonic
Product H	Sterimar Congestion Relief
Product K	Sterimar Stop and Protect Cold and Sinus Relief

The 6 solutions were diluted to an approximately 0.1% solution prior to analysis using ultra-pure filtered water to bring them into an acceptable range of 1000mg/L for ICP-MS analysis.

The samples were prepared in duplicate alongside a fully filtered sea water sample from Halen Mon. The seawater was collected from the Menai Straits off the coast of Anglesey (53°10’50”N 4°14’00”W).

Concentrations were obtained for the trace metals of interest. Fe^{3+} concentrations were found to be below the level of detection.

3.6.1.1 Cu^{2+} Analysis

The results for Cu^{2+} can be seen in Table 38. Values can be seen in ng/mL and µmol/L. The end column shows the Cu^{2+} concentration in a solution equivalent to an isotonic (0.9%) solution. Only 2 replicates were analysed, and this has given a high percentage standard deviation in products A and G. There is also a high percentage standard deviation for seawater where 6 replicates were analysed.

Table 38 - ICP-MS results for Cu^{2+} in ng/mL and µmol/L and in an equivalent 0.9% solution

	ng/ml	SD	µmol/L	SD	Conc in 0.9% solution ng/ml
Product A	19.71	8.63	0.31	0.14	11.82
Product B	12.27	0.49	0.19	0.01	12.27
Product E	14.45	0.14	0.23	0.00	14.45
Product G	42.35	8.11	0.67	0.13	19.06
Product H	367.77	13.17	5.79	0.21	165.49
Product K	930.83	13.22	14.65	0.21	418.87
Product SW	16.01	5.33	0.25	0.08	5.76

Figure 37 shows the concentration of Cu^{2+} in a 0.9% equivalent solution of each of the products. Visually it is possible to see that products H and K have a greater concentration of added Cu^{2+}. Product K contains 14 times the Cu^{2+}concentration than product A. These products state they have added Cu^{2+}.

All products show a higher concentration of Cu^{2+} than that of sea water. A one-way ANOVA analysis was performed on the concentrations of Cu^{2+}in all products. The results can be seen in Table 39. This shows that there are differences between the products with a p value of 6.17E-18.

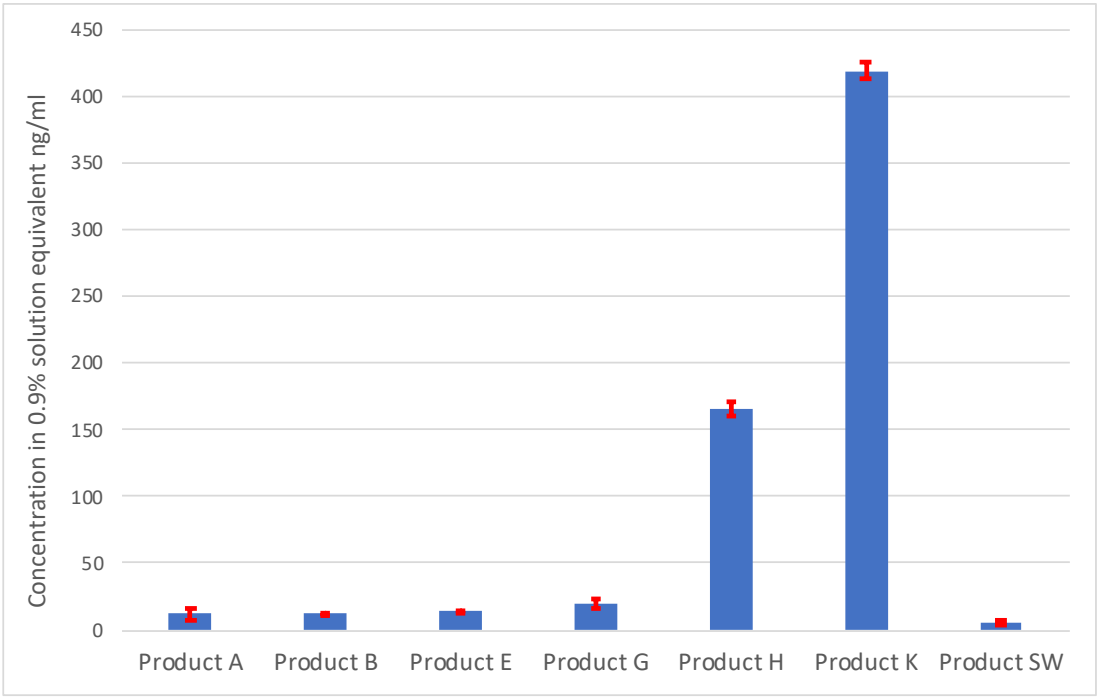


Figure 37 - Concentration of Cu^{2+} in a 0.9% equivalent solution of each of the products with the error bars showing SD

Table 39 – One-way ANOVA for Cu^{2+} in seawater and the seawater-based products at 0.9% solution

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Product A	2	23.65	11.82	26.84		
Product B	2	24.53	12.27	0.24		
Product E	2	28.89	14.45	0.02		
Product G	2	38.12	19.06	13.32		
Product H	2	330.99	165.49	35.11		
Product K	2	837.74	418.87	35.41		
Product SW	6	34.59	5.76	3.69		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	311027.05	6	51837.84	4407.83	6.17E-18	3.09
Within Groups	129.36	11	11.76			
Total	311156.41	17				

Products B and H state they are “enriched with copper” whereas product K has “added copper”. In Figure 37 it is difficult to see a difference in the Cu^{2+} concentration of B

(12.27 ng/mL) when compared with products A (11.82 ng/mL), E (14.45 ng/mL) and G (13.32 ng/mL), which made no mention of additives. Product B may not have had any additional Cu^{2+}. The phrase “enriched with” may have simply referred to the natural elements present in the sea water, or there may only have been a small amount added. Product K, which stated contains “added copper”, shows a concentration of 0.9 mg/L, a larger concentration of Cu^{2+} than any of the other products. A one-way ANOVA for products A to G can be seen in Table 40. This shows the concentrations are statistically similar with a p value of 0.23.

Table 40 - One-way ANOVA for Cu^{2+} in Products A-G at 0.9% solution

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Product A	2	23.65	11.82	26.84		
Product B	2	24.53	12.27	0.24		
Product E	2	28.89	14.45	0.02		
Product G	2	38.12	19.06	13.32		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	65.78	3	21.93	2.17	0.23	6.59
Within Groups	40.41	4	10.10			
Total	106.20	7				

3.6.1.2 Mn^{2+} Analysis

The results for Mn^{2+} can be seen in Table 41. Values can be seen in ng/mL and µmol/L. The end column shows the Cu^{2+} concentration in a solution equivalent to an isotonic (0.9%) solution. Only 2 replicates were analysed, and this gave a large standard deviation for Product E. Figure 38 shows the concentration of Mn^{2+} in a 0.9% equivalent solution of each of the products. Products A and H state that they contained “added Mn”. Product H showed an increased concentration and product A (3.27 ng/mL) showed a slight increase when compared to product E (2.53 ng/mL), however there was a large variance in the 2 replicates for Product E. as can be seen in Figure 38.

Table 41 - ICP-MS results for Mn^{2+} in ng/mL and µmol/L and in an equivalent 0.9% solution

	ng/ml	SD	µmol/L	SD	Conc in 0.9% solution ng/ml
Product A	5.46	0.61	0.10	0.01	3.27
Product B	12.73	0.24	0.23	0.00	12.73
Product E	6.94	6.33	0.13	0.12	6.94
Product G	5.63	0.88	0.10	0.02	2.53
Product H	14.00	1.26	0.25	0.02	6.30
Product K	8.07	0.33	0.15	0.01	3.63
Product SW	1.37	1.53	0.02	0.03	0.49

Product B showed a higher level of Mn^{2+} than was expected at 12.73 ng/mL, greater than in any other product. This was consistent across all replicates for product B. Mn^{2+} levels were higher in all products than in the locally sourced seawater. In general seawater, the concentration of Mn^{2+} is higher at 0.18 µmol/L.[70] Further analysis with more replicates was needed to get a clearer picture of Mn^{2+} concentration in all products.

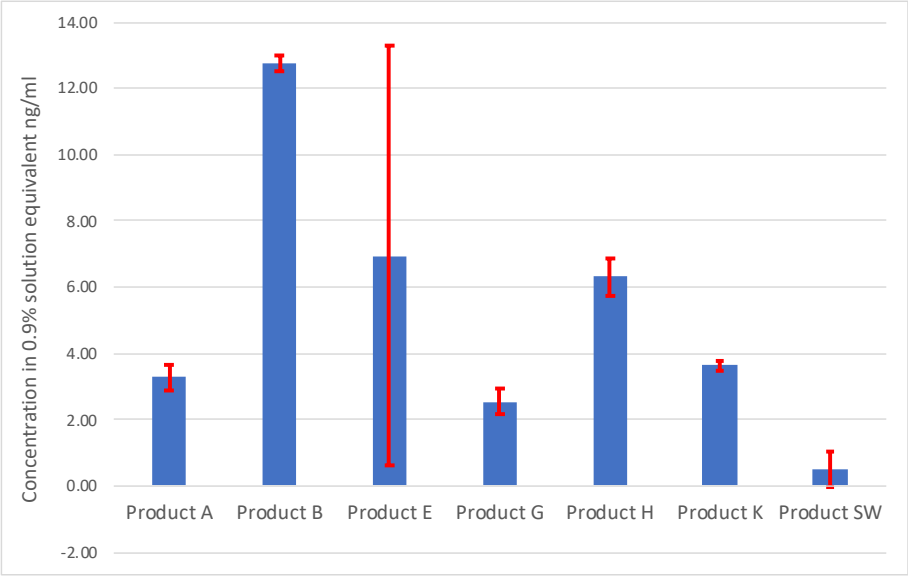


Figure 38 - Concentration of Mn^{2+} in a 0.9% equivalent solution of each of the products with the error bars showing SD

A one-way ANOVA analysis was performed on the concentrations of Mn^{2+} in all products. The detail can be seen in Table 42. This shows there are significant differences between the products.

Table 42 - One-way ANOVA for Mn^{2+} in SW and the seawater-based products at 0.9% solution

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Product A	2	6.55	3.27	0.14		
Product B	2	25.46	12.73	0.06		
Product E	2	13.89	6.94	40.01		
Product G	2	5.07	2.53	0.16		
Product H	2	12.60	6.30	0.32		
Product K	2	7.26	3.63	0.02		
Product SW	7	3.45	0.49	0.25		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	271.99	6	45.33	12.88	0.00013	3.00
Within Groups	42.23	12	3.52			
Total	314.22	18				

3.6.1.3 Zn^{2+} Analysis

The results for Zn^{2+} can be seen in Table 43. Values can be seen in ng/mL and µmol/L. The end column shows the Zn^{2+} concentration in a solution equivalent to an isotonic (0.9%) solution.

Figure 39 shows the concentration of Zn^{2+} in a 0.9% equivalent solution of each of the products. The Zn^{2+} concentration B (97.34 ng/mL), E (70.34 ng/mL), G (45.18 ng/mL) and H (43.59ng/mL). were higher than the values for seawater (17.71ng/mL). Zn^{2+} is found naturally in seawater at a concentration of Zn^{2+} nasal sprays have been reported to affect the sense of smell.[75]

Table 43 - ICP-MS results for Zn^{2+} in ng/mL and µmol/L and in an equivalent 0.9% solution

	ng/ml	SD	µmol/L	SD	Conc in 0.9% solution ng/ml
Product A	31.74	11.79	0.49	0.18	19.04
Product B	97.34	3.34	1.49	0.05	97.34
Product E	70.34	44.98	1.08	0.69	70.34
Product G	100.41	13.28	1.54	0.20	45.18
Product H	96.88	4.81	1.48	0.07	43.59
Product K	36.78	9.83	0.56	0.15	16.55
Product SW	49.19	13.49	0.63	0.36	17.71

A one-way ANOVA for all products, seen in Table 44 showed the values were significantly different (p value of 0.005), however looking at products A, K and seawater, the one-way ANOVA ,Table 45 shows these products were statistically similar (p-value of 0.78).

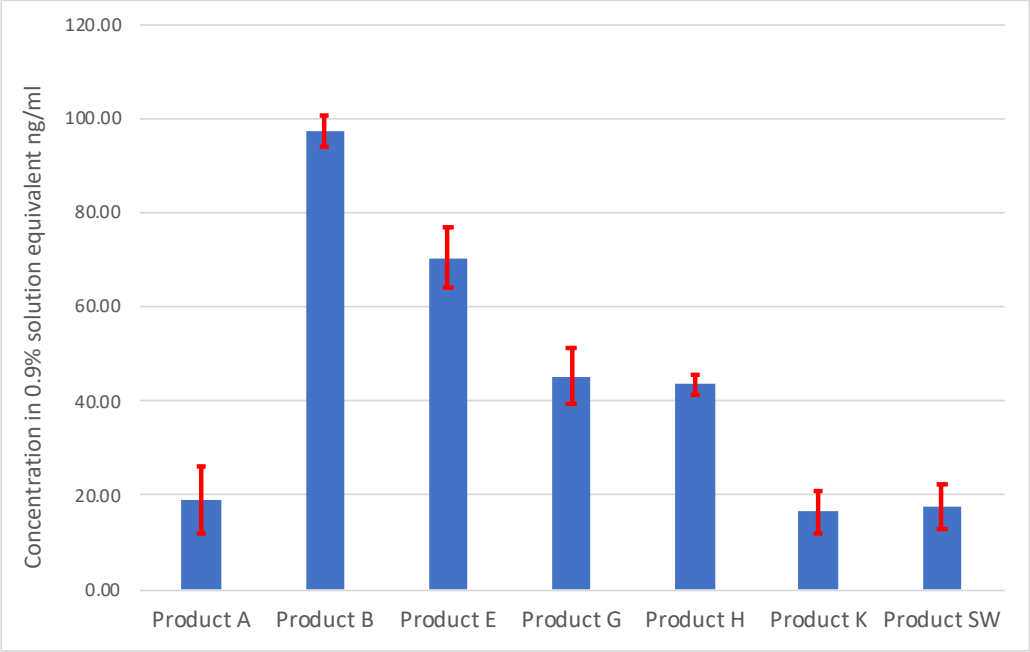


Figure 39 - Concentration of Zn^{2+} in a 0.9% equivalent solution of each of the products with the error bars showing SD

Table 44 - One-way ANOVA for Zn^{2+} in SW and the seawater-based products at 0.9% solution

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Product A	2	38.09	19.04	50.08		
Product B	2	194.67	97.34	11.12		
Product E	2	140.67	70.34	2023.06		
Product G	2	90.37	45.18	35.71		
Product H	2	87.19	43.59	4.69		
Product K	2	33.11	16.55	19.57		
Product SW	6	146.91	24.48	294.37		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11910.77	6	1985.13	6.04	0.005	3.09
Within Groups	3616.07	11	328.73			
Total	15526.84	17				

Table 45 - One-way ANOVA for Zn^{2+} in products A, K and seawater at 0.9% solution

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Product A	2	38.09	19.04	50.08		
Product K	2	33.11	16.55	19.57		
Product SW	6	146.91	24.48	294.37		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	113.52	2	56.76	0.26	0.78	4.74
Within Groups	1541.49	7	220.21			
Total	1655.01	9				

Further analysis was performed on the commercial products with a greater number of replicates. ICP-MS was able to quantify levels of trace metals in commercial products that were unable to be quantified with AAS or ICP-OES. As such, ICP-MS was determined to be the preferred option to analyse nasal mucus samples for trace metals.

3.6.2 Detailed Analysis of Trace Metal Content of Sea Water Based Nasal Products Analysed in section 3.6.1 using ICP-MS

The initial ICP-MS analysis in section 3.6.1 on the 6 seawater products gave promising results. Further analysis involving a greater number of replicates was required. Any outliers found are likely to be due to pipetting and dilution errors. The same 6 products, as listed in Table 37, and samples of Halen Môn seawater were prepared. Ten replicates of each product were tested to give a representative view of trace metal concentration. Al^{3+} was found to be present in nasal mucus samples (Section 6.1), therefore, Al^{3+} was added to the cations being analysed. Calibration charts for Al^{3+}, Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+} can be seen in Appendix H. The LOD and LOQ values can be seen in Table 46.

Table 46 - LOD and LOQ values for Al^{3+}, Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+}

	LoD ng/mL	LoQ ng/mL
Al^{3+}	0.03	1.48
Cu^{2+}	0.03	0.40
Fe^{3+}	0.51	4.65
Mn^{2+}	0.03	0.09
Zn^{2+}	0.04	0.41

The products and seawater samples were diluted to approximately a 0.1% solution for analysis. All results are quoted in both ng/mL and µmol/L. Concentrations of ions in seawater products are measured as % solution or mg/L. In nasal mucus, concentrations are recorded in mmol/L. Both units are required when looking at similarities and differences in the cation content of nasal mucus and the commercial nasal sprays. All analysis in this section has been performed using the analysis toolpak in Microsoft Excel or IBM SPSS.

3.6.2.1 Al^{3+} Analysis

Aluminium is not a trace metal found naturally in biological systems. Due to the levels of Al^{3+} found in nasal mucus, the commercial products and seawater samples were

tested for their Al^{3+} content. The results can be seen in Table 47 in ng/mL and in Table 48 in µmol/L. The concentrations can be seen visually in Figure 40 in µmol/L.

Table 47 - Al^{3+} concentrations in commercial products and seawater in ng/mL using analysis toolpak in Microsoft Excel

Al^{3+}	A	B	E	G	H	K	SW
Mean	116	<-0.00	58	<-0.00	<-0.00	55	<-0.00
Standard Error	2	<-0.00	2	<-0.00	<-0.00	3	<-0.00
Median	116	<-0.00	59	<-0.00	<-0.00	53	<-0.00
Standard Deviation	5	<-0.00	6	<-0.00	<-0.00	8	<-0.00
Sample Variance	28	<-0.00	40	<-0.00	<-0.00	67	<-0.00
Kurtosis	0.92	<-0.00	-0.44	<-0.00	<-0.00	2.53	<-0.00
Skewness	0.09	<-0.00	0.11	<-0.00	<-0.00	1.43	<-0.00
Range	19	<-0.00	21	<-0.00	<-0.00	29	<-0.00
Minimum	107	<-0.00	48	<-0.00	<-0.00	46	<-0.00
Maximum	126	<-0.00	69	<-0.00	<-0.00	74	<-0.00
Sum	1165	<-0.00	582	<-0.00	<-0.00	553	<-0.00
Count	10	10	10	10	10	10	10
Confidence Level(95.0%)	4	<-0.00	5	<-0.00	<-0.00	6	<-0.00
Lower Bound	113	<-0.00	54	<-0.00	<-0.00	49	<-0.00
Upper Bound	120	<-0.00	63	<-0.00	<-0.00	61	<-0.00

Table 48 - Al^{3+} concentrations in commercial products and seawater in µmol/L using analysis toolpak in Microsoft Excel

Al^{3+}	A	B	E	G	H	K	SW
Mean	4.3	<-0.00	2.2	<-0.00	<-0.00	2.1	<-0.00
Standard Error	0.1	<-0.00	0.1	<-0.00	<-0.00	0.1	<-0.00
Median	4.3	<-0.00	2.2	<-0.00	<-0.00	2.0	<-0.00
Standard Deviation	0.2	<-0.00	0.2	<-0.00	<-0.00	0.3	<-0.00
Sample Variance	0.0	<-0.00	0.1	<-0.00	<-0.00	0.1	<-0.00
Kurtosis	0.92	<-0.00	-0.44	<-0.00	<-0.00	2.53	<-0.00
Skewness	0.09	<-0.00	0.11	<-0.00	<-0.00	1.43	<-0.00
Range	0.7	<-0.00	0.8	<-0.00	<-0.00	1.1	<-0.00
Minimum	4.0	<-0.00	1.8	<-0.00	<-0.00	1.7	<-0.00
Maximum	4.7	<-0.00	2.5	<-0.00	<-0.00	2.8	<-0.00
Sum	43.2	<-0.00	21.6	<-0.00	<-0.00	20.5	<-0.00
Count	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Confidence Level(95.0%)	0.1	<-0.00	0.2	<-0.00	<-0.00	0.2	<-0.00
Lower Bound	4.2	<-0.00	2.0	<-0.00	<-0.00	1.8	<-0.00
Upper Bound	4.5	<-0.00	2.3	<-0.00	<-0.00	2.3	<-0.00

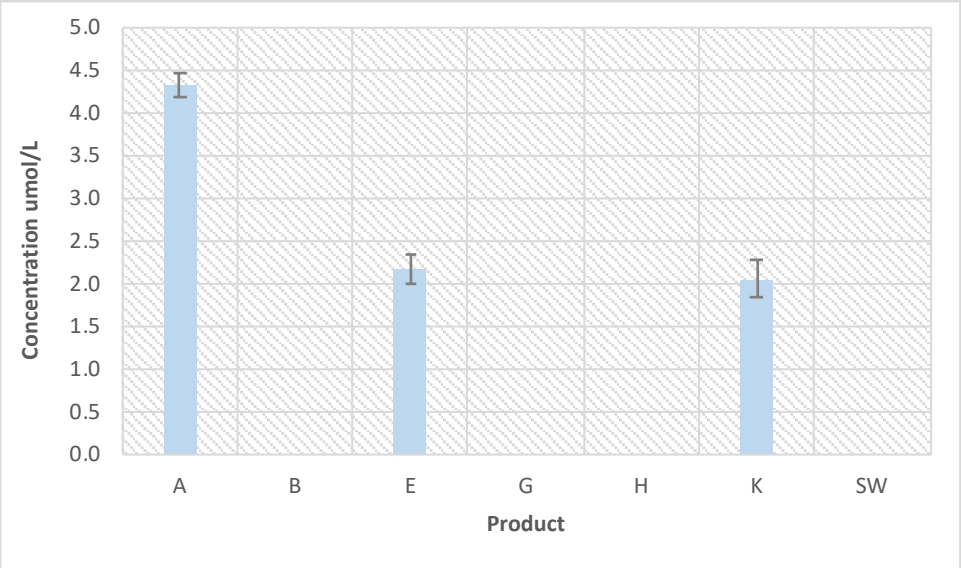


Figure 40 - Al^{3+} concentrations in commercial products with the upper and lower bound limits

Small amounts of Al^{3+} can be seen in 3 of the products. Visually products E and K look similar. A one-way ANOVA gave a p-value of 0.39. This can be seen in Table 49.It is unclear if the Al^{3+} content found in the products was a contaminant, possibly from the metal in the spray mechanism, as it is not present in all products of the same brand, or if it is present in the product. Al^{3+} was below the LOD in the seawater samples.

Table 49 - One-way ANOVA for Al^{3+} in products E & K using Microsoft Excel Analysis Toolpak

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Product E	10	21.59	2.16	0.06		
Product K	10	20.51	2.05	0.09		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.06	1	0.06	0.78	0.39	4.41
Within Groups	1.33	18	0.07			
Total	1.39	19				

3.6.2.2 Cu^{2+} Analysis

Cu^{2+} is found in seawater[70], and a proportionate amount should be found in the seawater products. The test analysis, using 2 replicates, found Cu^{2+} concentrations in all products, section 3.6.1. To get a clearer picture, 10 replicates of each product were analysed. The results in ng/mL can be seen in Table 50.

Table 50 - Cu^{2+} concentrations in commercial products and seawater in ng/mL

Cu^{2+}	A	B	E	G	H	K	SW
Mean	27.7	7.2	8.8	13.1	492.1	949.8	23.3
Standard Error	2.7	0.2	0.9	0.7	3.1	9.3	1.9
Median	25.7	6.9	7.9	12.4	490.1	945.1	22.1
Standard Deviation	8.5	0.7	2.9	2.1	9.8	29.3	5.9
Sample Variance	71.6	0.5	8.1	4.6	97.0	860.0	34.3
Kurtosis	3.02	-1.11	5.34	6.53	0.74	2.12	3.52
Skewness	1.52	0.72	2.22	2.50	0.85	1.25	1.75
Range	29.4	1.9	9.5	7.2	33.6	99.7	19.8
Minimum	18.4	6.4	6.6	11.5	478.9	916.3	17.6
Maximum	47.7	8.3	16.1	18.7	512.5	1016.1	37.4
Sum	277.2	72.0	87.9	130.5	4921.2	9497.9	232.7
Count	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Confidence Level(95.0%)	6.1	0.5	2.0	1.5	7.0	21.0	4.2
Lower Bound	21.7	6.7	6.7	11.5	485.1	928.8	19.1
Upper Bound	33.8	7.7	10.8	14.6	499.2	970.8	27.5

The kurtosis values for products A, E, G and SW are above 3, the dataset has heavier tails than a normal distribution. The distribution is leptokurtic, with more values close to the mean. There is a greater likelihood of more outliers. Products B, H and K have a kurtosis value less than 3. These have a platykurtic distribution, there is a less likely chance of extreme outliers. Outlier analysis was performed on the commercial product dataset using SPSS. There was one outlier in product A, one in product E, two on product G and one in the seawater dataset. These can be seen visually in Figure 41.

The outlier values were removed from the dataset. The results can be seen in Table 51 in ng/mL and in Table 52 in µmol/L. Product B does not show an increased concentration of Cu^{2+}. The packaging for product B indicates the product is enriched with Cu^{2+}, but any addition is not quantified.

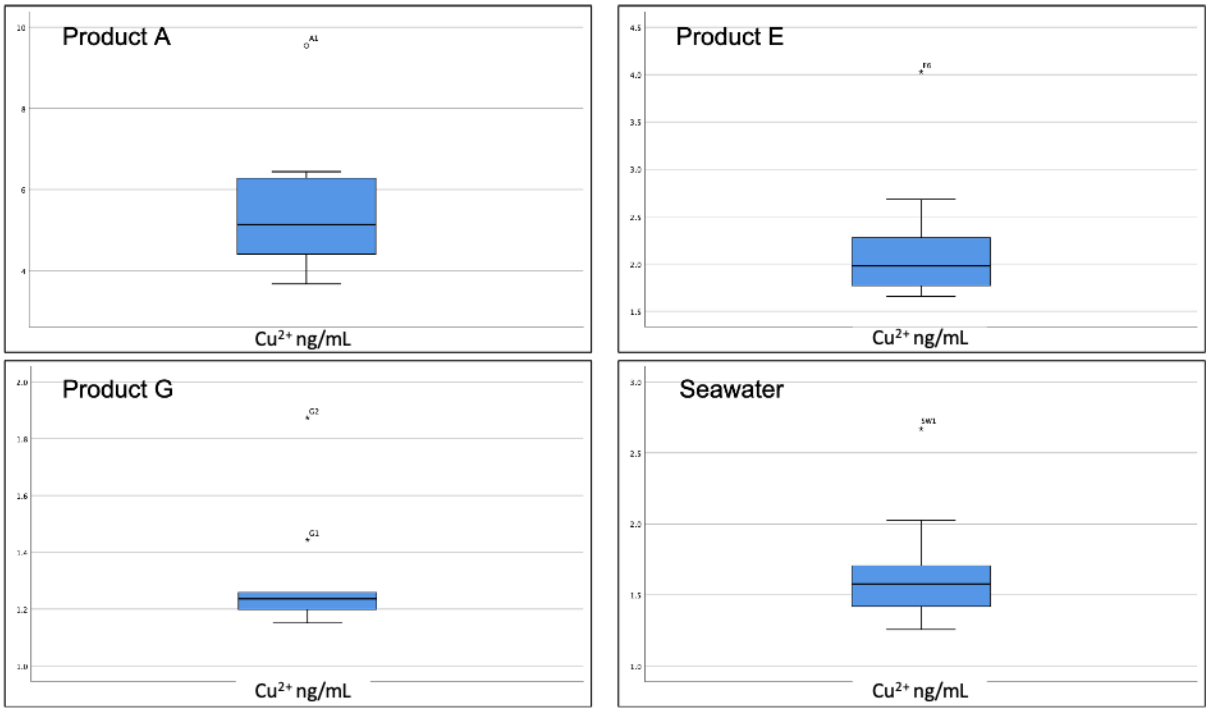


Figure 41 - Outlier analysis for Cu^{2+} displaying the outliers in Product A, Product E, Product G and seawater samples

Products H and K have demonstrable high levels Cu^{2+} present. Product K states it has added Cu^{2+} and H is enriched with Cu^{2+}. Results indicate these products contain 20 and 50 times the Cu^{2+} concentration to the seawater samples respectively.

Table 51 - Cu^{2+} concentrations in commercial products and seawater in ng/mL minus outlier values

Cu^{2+}	*A*	*B*	*E*	*G*	*H*	*K*	*SW*
Mean	25.5	7.2	2.0	1.2	492.1	949.8	1.6
Standard Error	1.7	0.2	0.1	0.0	3.1	9.3	0.1
Median	24.7	6.9	2.0	1.2	490.1	945.1	1.6
Standard Deviation	5.0	0.7	0.3	0.0	9.8	29.3	0.2
Sample Variance	25.0	0.5	0.1	0.0	97.0	860.0	0.1
Kurtosis	-1.43	-1.11	1.38	-0.53	0.74	2.12	1.10
Skewness	0.07	0.72	1.22	-0.66	0.85	1.25	0.78
Range	13.8	1.9	1.0	0.1	33.6	99.7	0.8
Minimum	18.4	6.4	1.7	1.2	478.9	916.3	1.3
Maximum	32.2	8.3	2.7	1.3	512.5	1016.1	2.0
Sum	229.5	72.0	17.9	9.7	4921.2	9497.9	14.0
Count	9.0	10.0	9.0	8.0	10.0	10.0	9.0
Confidence Level(95.0%)	3.8	0.5	0.3	0.0	7.0	21.0	0.2
Lower Bound	21.6	6.7	1.7	1.2	485.1	928.8	1.4
Upper Bound	29.3	7.7	2.2	1.2	499.2	970.8	1.7

Table 52 - Cu^{2+} concentrations in commercial products and seawater in umol/L minus outlier values

Cu^{2+}	A	B	E	G	H	K	SW
Mean	0.40	0.11	0.13	0.19	7.74	14.95	0.34
Standard Error	0.03	0.00	0.01	0.00	0.05	0.15	0.02
Median	0.39	0.11	0.12	0.19	7.71	14.87	0.35
Standard Deviation	0.08	0.01	0.02	0.01	0.15	0.46	0.05
Sample Variance	0.01	0.00	0.00	0.00	0.02	0.21	0.00
Kurtosis	-1.43	-1.11	1.38	-0.53	0.74	2.12	1.10
Skewness	0.07	0.72	1.22	-0.66	0.85	1.25	0.78
Range	0.22	0.03	0.06	0.02	0.53	1.57	0.17
Minimum	0.29	0.10	0.10	0.18	7.54	14.42	0.28
Maximum	0.51	0.13	0.17	0.20	8.07	15.99	0.45
Sum	3.61	1.13	1.13	1.53	77.44	149.47	3.07
Count	9	10	9	8	10	10	9
Confidence Level(95.0%)	0.06	0.01	0.02	0.01	0.11	0.33	0.04
Lower Bound	0.34	0.11	0.11	0.19	7.63	14.62	0.30
Upper Bound	0.46	0.12	0.14	0.20	7.86	15.28	0.38

The concentrations of Cu^{2+} can be seen visually in Figure 42. The chart shows the varied concentration of Cu^{2+} across the products.

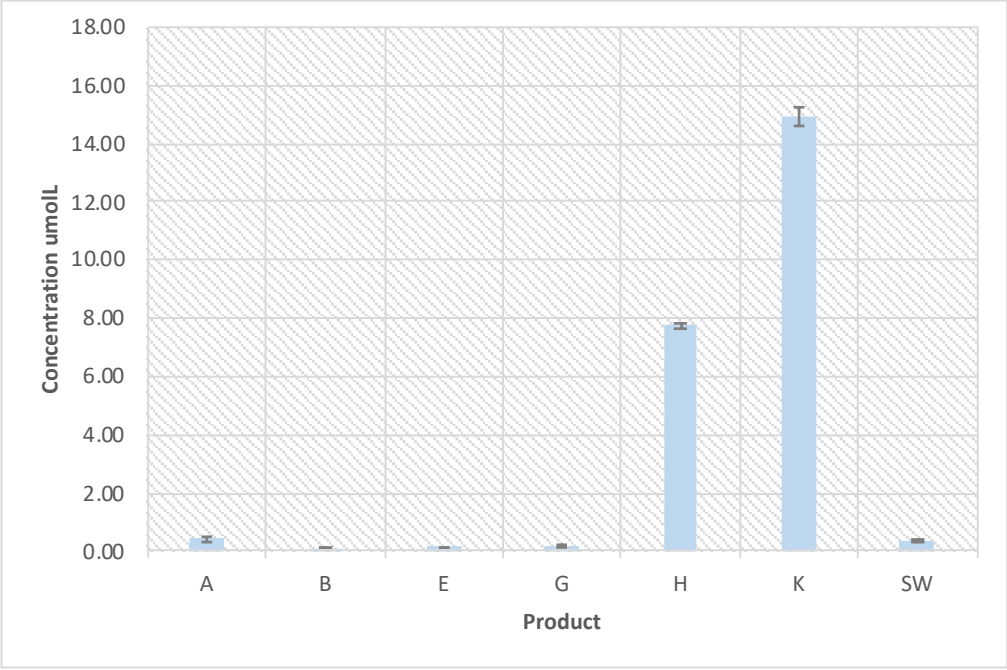


Figure 42 – Visual representation of Cu^{2+} concentrations in commercial products with the upper and lower bound limits shown by error bars

3.6.2.3 Mn^{2+} Analysis

Mn^{2+} is found in small quantities in seawater. The results including any outliers can be seen in Table 53 in ng/mL.

Table 53 - Mn^{2+} concentrations in commercial products and Halen Mon seawater in ng/mL

Mn^{2+}	*A*	*B*	*E*	*G*	*H*	*K*	*SW*
Mean	3.6	12.4	4.5	5.7	6.2	6.4	0.7
Standard Error	0.1	0.1	0.1	0.2	0.1	0.2	0.0
Median	3.5	12.4	4.6	5.6	6.2	6.4	0.7
Standard Deviation	0.4	0.3	0.2	0.5	0.3	0.6	0.1
Sample Variance	0.1	0.1	0.0	0.3	0.1	0.3	0.0
Kurtosis	4.20	-0.43	-0.22	3.65	-0.48	-0.46	-1.60
Skewness	1.73	0.82	-0.25	1.66	0.16	0.27	-0.20
Range	1.4	0.9	0.7	1.8	1.1	1.8	0.4
Minimum	3.1	12.1	4.2	5.2	5.6	5.6	0.5
Maximum	4.5	13.0	4.9	7.0	6.8	7.4	0.9
Sum	35.8	124.5	45.3	57.0	61.7	64.4	6.9
Count	10	10	10	10	10	10	10
Confidence Level(95.0%)	0.3	0.2	0.2	0.4	0.2	0.4	0.1
Lower Bound	3.3	12.2	4.4	5.3	5.9	6.0	0.6
Upper Bound	3.8	12.7	4.7	6.1	6.4	6.8	0.8

The kurtosis values for products A and G indicate there may be outliers present in the dataset, Outlier analysis was performed using SPSS. The outliers can be seen in the box plot diagrams in Figure 43.

Figure 43 - Outlier analysis for Mn^{2+} displaying the outliers in Product A and Product G samples

Both product A and product B contained one outlier each and were removed from the dataset. The results minus the outliers can be seen in Table 54 in ng/mL and Table

55 in µmol/L. In standard seawater, there is 0.18 µmol/L Mn^{2+}. The seawater off the coast of Anglesey is recording 1/10th of this value.

Table 54 - Mn^{2+} concentrations in commercial products and Halen Mon seawater in ng/mL minus outliers

Mn^{2+}	A	B	E	G	H	K	SW
Mean	3.48	12.45	4.53	5.56	6.17	6.44	0.69
Standard Error	0.07	0.09	0.07	0.10	0.11	0.18	0.04
Median	3.51	12.35	4.61	5.55	6.17	6.43	0.70
Standard Deviation	0.20	0.29	0.22	0.30	0.34	0.57	0.13
Sample Variance	0.04	0.09	0.05	0.09	0.11	0.32	0.02
Kurtosis	0.20	-0.43	-0.22	-1.37	-0.48	-0.46	-1.60
Skewness	-0.27	0.82	-0.25	-0.05	0.16	0.27	-0.20
Range	0.67	0.89	0.73	0.81	1.12	1.77	0.35
Minimum	3.13	12.09	4.17	5.17	5.64	5.60	0.51
Maximum	3.80	12.98	4.90	5.98	6.76	7.37	0.86
Sum	31.33	124.46	45.34	50.03	61.71	64.38	6.91
Count	9.00	10.00	10.00	9.00	10.00	10.00	10.00
Confidence Level(95.0%)	0.15	0.21	0.15	0.23	0.24	0.40	0.09
Lower Bound	3.33	12.24	4.38	5.32	5.93	6.03	0.60
Upper Bound	3.63	12.66	4.69	6.09	6.41	6.84	0.78

Table 55 - Mn^{2+} concentrations in commercial products and Halen Mon seawater in umol/L minus outliers

Mn^{2+}	A	B	E	G	H	K	SW
Mean	0.06	0.23	0.08	0.10	0.11	0.12	0.01
Standard Error	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Median	0.06	0.22	0.08	0.10	0.11	0.12	0.01
Standard Deviation	0.00	0.01	0.00	0.01	0.01	0.01	0.00
Sample Variance	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kurtosis	0.20	-0.43	-0.22	-1.37	-0.48	-0.46	-1.60
Skewness	-0.27	0.82	-0.25	-0.05	0.16	0.27	-0.20
Range	0.01	0.02	0.01	0.01	0.02	0.03	0.01
Minimum	0.06	0.22	0.08	0.09	0.10	0.10	0.01
Maximum	0.07	0.24	0.09	0.11	0.12	0.13	0.02
Sum	0.57	2.27	0.83	0.91	1.12	1.17	0.13
Count	9.00	10.00	10.00	9.00	10.00	10.00	10.00
Confidence Level(95.0%)	0.003	0.004	0.003	0.004	0.004	0.007	0.002
Lower Bound	0.06	0.22	0.08	0.10	0.11	0.11	0.01
Upper Bound	0.07	0.23	0.09	0.11	0.12	0.12	0.01

The concentrations of Mn^{2+} can be seen visually in Figure 44.

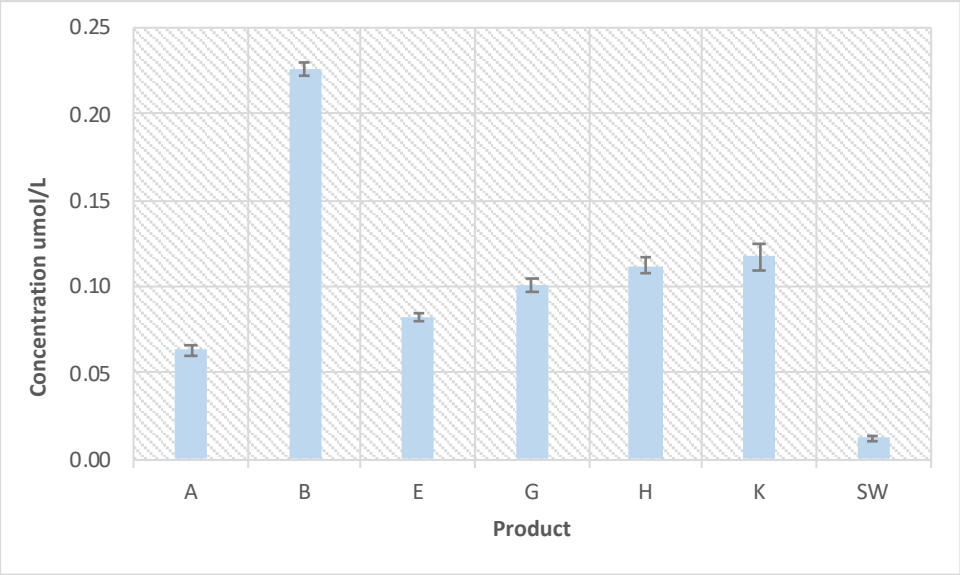


Figure 44 – Visual representation of Mn^{2+} concentrations in commercial products with the upper and lower bound limits shown by error bars

Product A gave a lower level of Mn^{2+} (3.48 ng/mL) than the other products in Table 54. Despite the product packaging stating it had added Mn^{2+}. One-way ANOVA analysis was performed on products of a 0.9% solution equivalence to see if the products were statistically similar. The p value was 4.33E-75 showing the products were statistically different as seen in Table 56.

Table 56 - One-way ANOVA for in Mn^{2+} in all products at a 0.9% solution equivalence using Microsoft Excel Analysis Toolpak

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Product A	10	21.49	2.15	0.05		
Product B	10	124.46	12.45	0.09		
Product E	10	45.34	4.53	0.05		
Product G	10	25.66	2.57	0.06		
Product H	10	24.15	2.41	0.02		
Product J	10	25.19	2.52	0.05		
Product K	10	38.42	3.84	0.02		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	809.68	6	134.95	2931.75	4.33E-75	2.25
Within Groups	2.90	63	0.05			
Total	812.58	69				

A one-way ANOVA was performed on the products with similar concentrations to test for significant similarities. The results can be seen in Table 57. The p value of 0.0003 indicates that they are significantly different.

Table 57 - One-way ANOVA for in Mn^{2+} in all products A, G, H and J at a 0.9% solution equivalence using Microsoft Excel Analysis Toolpak

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Product A	10	21.49	2.15	0.05		
Product G	10	25.66	2.57	0.06		
Product H	10	24.15	2.41	0.02		
Product J	10	25.19	2.52	0.05		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.04	3	0.35	8.03	0.0003	2.87
Within Groups	1.56	36	0.04			
Total	2.60	39				

3.6.2.4 Zn^{2+} Analysis

The concentration of Zn^{2+} in the 6 commercial products and Halen Mon seawater can be seen in Table 58 in ng/mL

The kurtosis for product G has a value greater than 3, meaning there is a greater likelihood of extreme outliers. Outlier analysis was performed in SPSS. The results can be seen in the box plots in Figure 45.

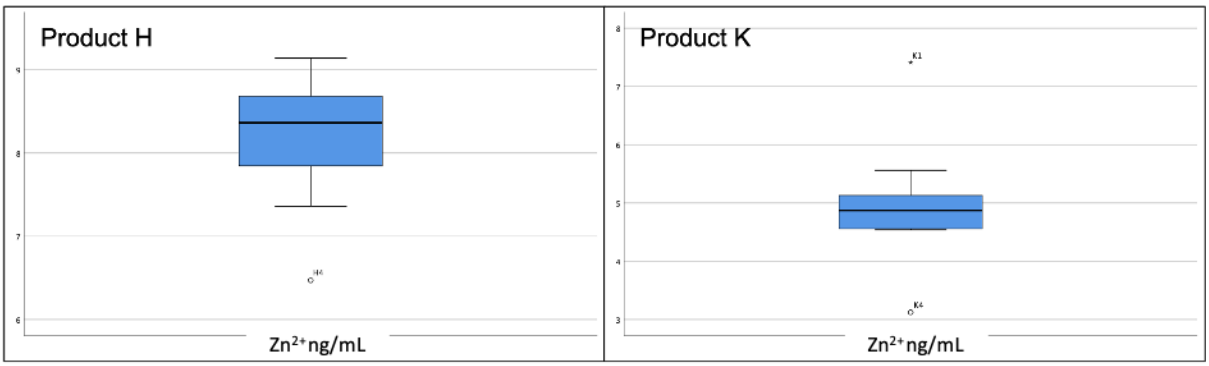


Figure 45 - Outlier analysis for Zn^{2+} displaying the outliers in Product H and Product K samples

Table 58 - Zn^{2+} concentrations in commercial products and Halen Mon seawater in ng/mL

Zn^{2+}	A	B	E	G	H	K	SW
Mean	27.5	105.0	70.0	80.6	82.0	49.6	48.9
Standard Error	1.2	1.1	0.9	5.2	2.6	3.4	3.2
Median	27.1	105.1	70.6	77.3	83.6	48.7	52.7
Standard Deviation	3.8	3.4	2.9	16.4	8.1	10.7	10.2
Sample Variance	14.1	11.9	8.3	270.5	65.3	114.1	103.7
Kurtosis	-0.60	0.05	-1.77	-0.67	1.31	3.68	-1.26
Skewness	0.50	-0.14	-0.13	0.81	-1.10	0.97	-0.57
Range	10.7	11.4	7.9	44.8	26.7	42.9	27.5
Minimum	22.8	99.6	65.9	65.1	64.7	31.2	33.6
Maximum	33.5	111.0	73.8	109.8	91.4	74.2	61.1
Sum	274.9	1050.4	699.8	806.2	820.4	496.4	488.6
Count	10	10	10	10	10	10	10
Confidence Level(95.0%)	2.7	2.5	2.1	11.8	5.8	7.6	7.3
Lower Bound	24.8	102.6	67.9	68.9	76.3	42.0	41.6
Upper Bound	30.2	107.5	72.0	92.4	87.8	57.3	56.1

Outlier analysis found one outlier in product H and 2 in product K. The outliers in product K were more extreme. The data excluding the outliers can be seen in Table 59 in ng/mL and in Table 60 in µmol/L.

Table 59 - Zn^{2+} concentrations in commercial products and Halen Mon seawater in ng/mL minus outliers

Zn^{2+}	A	B	E	G	H	K	SW
Mean	27.5	105.0	70.0	80.6	84.0	48.9	48.9
Standard Error	1.2	1.1	0.9	5.2	1.9	1.2	3.2
Median	27.1	105.1	70.6	77.3	84.2	48.7	52.7
Standard Deviation	3.8	3.4	2.9	16.4	5.6	3.4	10.2
Sample Variance	14.1	11.9	8.3	270.5	31.7	11.7	103.7
Kurtosis	-0.60	0.05	-1.77	-0.67	0.24	1.11	-1.26
Skewness	0.50	-0.14	-0.13	0.81	-0.44	1.05	-0.57
Range	10.7	11.4	7.9	44.8	17.8	10.2	27.5
Minimum	22.8	99.6	65.9	65.1	73.6	45.4	33.6
Maximum	33.5	111.0	73.8	109.8	91.4	55.6	61.1
Sum	274.9	1050.4	699.8	806.2	755.7	391.0	488.6
Count	10	10	10	10	9	8	10
Confidence Level(95.0%)	2.7	2.5	2.1	11.8	4.3	2.9	7.3
Lower Bound	24.8	102.6	67.9	68.9	79.6	46.0	41.6
Upper Bound	30.2	107.5	72.0	92.4	88.3	51.7	56.1

Table 60 - Zn^{2+} concentrations in commercial products and Halen Mon seawater in umol/L minus outliers

Zn^{2+}	A	B	E	G	H	K	SW
Mean	0.42	1.61	1.07	1.23	1.28	0.75	0.75
Standard Error	0.02	0.02	0.01	0.08	0.03	0.02	0.05
Median	0.41	1.61	1.08	1.18	1.29	0.75	0.81
Standard Deviation	0.06	0.05	0.04	0.25	0.09	0.05	0.16
Sample Variance	0.00	0.00	0.00	0.06	0.01	0.00	0.02
Kurtosis	-0.60	0.05	-1.77	-0.67	0.24	1.11	-1.26
Skewness	0.50	-0.14	-0.13	0.81	-0.44	1.05	-0.57
Range	0.16	0.17	0.12	0.68	0.27	0.16	0.42
Minimum	0.35	1.52	1.01	1.00	1.13	0.69	0.51
Maximum	0.51	1.70	1.13	1.68	1.40	0.85	0.93
Sum	4.21	16.07	10.70	12.33	11.56	5.98	7.47
Count	10.00	10.00	10.00	10.00	9.00	8.00	10.00
Confidence Level(95.0%)	0.04	0.04	0.03	0.18	0.07	0.04	0.11
Lower Bound	0.38	1.57	1.04	1.05	1.22	0.70	0.64
Upper Bound	0.46	1.64	1.10	1.41	1.35	0.79	0.86

These values can be seen visually in Figure 46.

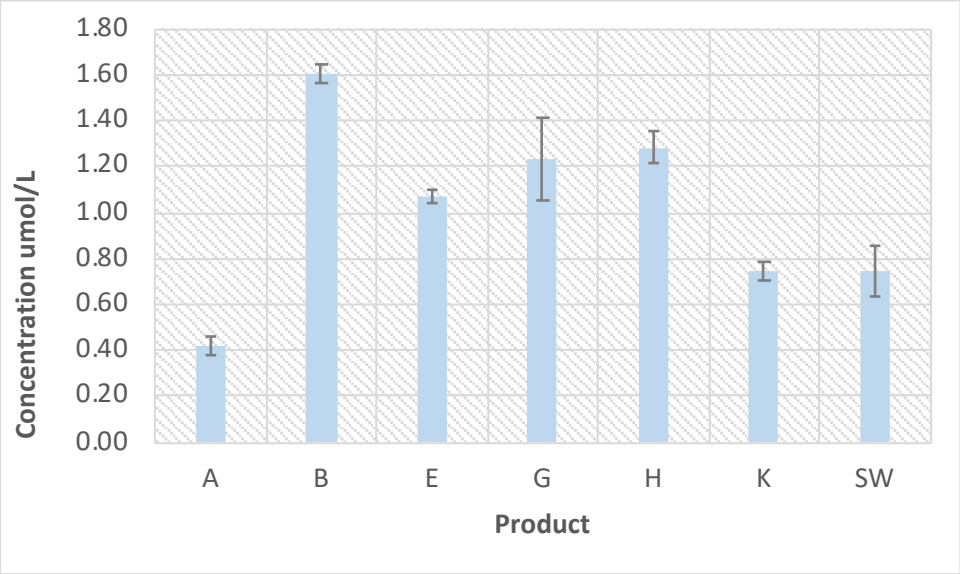


Figure 46 – Visual representation of Zn^{2+} concentrations in commercial products with the upper and lower bound limits

The concentration of Zn^{2+} in general seawater has great variability.[76] The values can fluctuate. The varied values seen in the products may be down to the location, time of year or the depth at which the water was harvested. The product packaging does not indicate any added Zn^{2+}.

3.7 **Conclusion Nasal Wash Products**

The nasal sprays currently on the market do not have to list the ingredients contained in the product. The results from ICP-MS analysis 2 and AAS analysis one can be pulled together to get a total view for each product. There were a small number of outliers identified in the results. During preparation, samples were serial diluted, using micro volumes of each product in 50 mL of ultra-pure water *via* micro pipette. The outliers found are likely to be due to pipetting and dilution errors. The total results for all products can be seen in Table 61 in mg/L and in Table 62 in mmol/L. The mean Na^+ value for seawater is lower than expected. Seawater has a value of approximately 10500 mg/L. This value falls close to the upper bound value 10465 mg/L in Table 24. Products B and E are made by the same company, Sterimar, and apart from the packaging, they are essentially the same. Product B is targeted as a cold defence product that is enriched with Cu^{2+} and product E is a nasal hygiene spray that can be used daily to maintain nasal health. Both products state that they are rich in sea minerals. The numbers suggest they are purely diluted seawater. Product H, Sinomarin Hypertonique states that it is a 2.3% NaCl solution. The results show a mean value of 2.35% for the product. The product does not claim to contain any other additives and the results back this up. Product A is a mildly hypertonic at a 1.5% solution. The packaging claims it has added Ca^{2+} and Mn^{2+}. In none of the analysis has a greater concentration of Mn^{2+} been found than in any of the isotonic products. There is approximately 0.5 mg/L of added Ca^{2+} in the product. The other 2 remaining products product H, Sterimar Congestion Relief and Product K, Sterimar Stop and Protect Cold and Sinus Relief, are both labelled as hypertonic and have similar % solutions at approximately 2.25%. Product H states it contains an added Cu^{2+} salt. This is evident in the concentration at almost 0.5mg/L. Product K also claims added copper. This product contains 0.9 mg/L of Cu^{2+}.

The osmolality of the products is also consistent with the % solution values seen. The pH of the products is also important. The pH's seen indicates that the pH needs to be close to neutral, pH 7. Sea water is usually more basic. The commercial products indicate that they are buffered to give a neutral pH.

Table 61 - Concentrations of ions in 6 products and seawater samples in mg/L

		Na^+	SD	K^+	SD	Mg^{2+}	SD	Ca^{2+}	SD	Al^{3+}	SD	Cu^{2+}	SD	Mn^{2+}	SD	Zn^{2+}	SD
Product A	Sterimar Stop & Protect Allergy Response	5977.79	485.38	225.36	4.81	577.17	11.65	723.54	8.70	0.12	0.01	0.0255	0.0050	0.0035	0.0002	0.027	0.004
Product B	Sterimar Cold Defense	3527.28	253.68	135.63	4.05	354.58	8.44	181.66	3.43	<0.00	<0.00	0.0072	0.0007	0.0124	0.0003	0.105	0.003
Product E	Sterimar Isotonic Nasal Hygiense Spray	3475.79	415.85	137.06	2.92	336.44	10.36	157.95	4.63	0.06	0.01	0.0020	0.0003	0.0045	0.0002	0.070	0.003
Product G	Sinomarin Hypertonique	9250.61	935.44	312.42	9.46	824.67	21.42	353.96	3.20	<0.00	<0.00	0.0012	0.0000	0.0056	0.0003	0.081	0.016
Product H	Sterimar Congestion Relief	8739.89	611.62	330.80	5.30	877.72	31.09	358.33	9.24	<0.00	<0.00	0.4921	0.0098	0.0062	0.0003	0.084	0.006
Product K	Sterimar Stop and Protect Cold and Sinus Relief	8858.99	361.77	348.30	11.44	890.35	29.87	345.35	16.08	0.06	0.01	0.9498	0.0293	0.0064	0.0006	0.049	0.003
SW	Halen Mon Seawater	9604.97	1200.98	416.69	10.39	1106.62	22.43	411.35	8.55	<0.00	<0.00	0.0016	0.0002	0.0007	0.0001	0.049	0.010

Table 62 - Concentrations of ions in 6 products and seawater samples in mmol/

		Na^+	SD	K^+	SD	Mg^{2+}	SD	Ca^{2+}	SD	Al^{3+}	SD	Cu^{2+}	SD	Mn^{2+}	SD	Zn^{2+}	SD
Product A	Sterimar Stop & Protect Allergy Response	260.02	21.11	5.76	0.12	23.75	0.48	18.05	0.22	0.0043	0.0002	0.00040	0.00008	0.00006	0.00000	0.0004	0.0001
Product B	Sterimar Cold Defense	153.43	11.03	3.47	0.10	14.59	0.35	4.53	0.09	<0.0000	<0.0000	0.00011	0.00001	0.00023	0.00001	0.0016	0.0001
Product E	Sterimar Isotonic Nasal Hygiense Spray	151.19	18.09	3.51	0.07	13.84	0.43	3.94	0.12	0.0022	0.0002	0.00013	0.00002	0.00008	0.00000	0.0011	0.0000
Product G	Sinomarin Hypertonique	402.38	40.69	7.99	0.24	33.93	0.88	8.83	0.08	<0.0000	<0.0000	0.00019	0.00001	0.00010	0.00001	0.0012	0.0003
Product H	Sterimar Congestion Relief	380.16	26.60	8.46	0.14	36.11	1.28	8.94	0.23	<0.0000	<0.0000	0.00774	0.00015	0.00011	0.00001	0.0013	0.0001
Product K	Sterimar Stop and Protect Cold and Sinus Relief	385.34	15.74	8.91	0.29	36.63	1.23	8.62	0.40	0.0021	0.0003	0.01495	0.00046	0.00012	0.00001	0.0007	0.0001
SW	Halen Mon Seawater	417.79	52.24	10.66	0.27	45.53	0.92	10.26	0.21	<0.0000	<0.0000	0.00034	0.00005	0.00001	0.00000	0.0007	0.0002

3.8 Experimental – IC Analysis Nasal Products

All chemicals were purchased from Fisher Scientific or Sigma Aldrich and used as directed and all analysis carried out used a Dionex ICS2100 Ion Chromatography machine

3.8.1 Exploratory Nasal Wash Sample Bulk Metal Analysis

Calibration standards of 200 mg/L, 100 mg/L, 50 mg/L, 20 mg/L, 10 mg/L, 5 mg/L and 1 mg/L (based on Na^+ content) were prepared from a Dionex multi-ion cation IC standard (Na^+ 200 mg/L, Mg^{2+} 250 mg/L, Li^+ 50 mg/L, NH_4^+ 250 mg/L, Ca^{2+} 500 mg/L and K^+ 500 mg/L), concentrations in Table 63.

Table 63 - Calibration standard concentrations using 6-ion cation standard concentration in mg/L

Ion	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7
Na^+	1	5	10	20	50	100	200
K^+	2.5	12.5	25	50	125	250	500
Mg^{2+}	1.25	6.25	12.5	25	62.5	125	250
Ca^{2+}	2.5	12.5	25	50	125	250	500

Dilute samples of an isotonic nasal sprays were analysed. Isotonic nasal sprays are typically a 0.9% saline solution, meaning there are 9 g of sodium chloride (NaCl) in 1 litre giving a Na^+ concentration of approximately 3.5 g/L. Based on this number, a stock solution of approximately 200 mg/L was made, from which, samples ranging from approximately 1 mg/L to 200 mg/L were prepared.

3.8.2 Nasal Spray 10 product initial Bulk Metal Analysis.

Calibration standards of 100 mg/L, 75 mg/L, 50 mg/L, 20 mg/L, 10 mg/L and 5 mg/L (based on Na^+ content) were prepared from a Dionex multi-ion cation IC standard (Na^+ 200 mg/L, Mg^{2+} 250 mg/L, Li^+ 50 mg/L, NH_4^+ 250 mg/L, Ca^{2+} 500 mg/L and K^+ 500 mg/L), concentrations in Table 64.

Table 64 - Calibration standard concentrations using 6-ion cation standards for the analysis of the initial 10 products. Concentrations in mg/L

Ion	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6
Na^+	5	10	20	50	75	100
K^+	12.5	25	50	125	187.5	250
Mg^{2+}	6.25	12.5	25	62.5	93.75	125
Ca^{2+}	12.5	250	50	125	187.5	250

Sample solutions were prepared to an estimated 100 mg/L and 50 mg/L. In total 80 samples were tested, 8 for each product to include 2 different batches with replicates. NeilMed Sinus Wash and Himalayan Salt were prepared with 240 mL of deionised water as per the packaging instructions prior to dilution.

3.8.3 **Nasal Spray 10 product Optimised Bulk Metal Analysis.**

Sample solutions were prepared to an estimated 100 mg/L based on the estimated Na^+ content. In total, 40 samples were analysed, 10 products, 2 batches and replicates. Samples of Halen Môn salt, spa salt (unwashed), pure salt (washed), 0.1 micron filtered sea water, and fully filtered seawater, were prepared for analysis. 2 g salt (equivalent mass to Himalayan salt) was dissolved in water prior to dilution and the sea water samples were diluted to approx. 100 mg/L.

Individual calibration standards were used to enable calibration at lower concentrations for K^+, Mg^{2+} and Ca^{2+}. The 10 selected products were run in duplicate from 2 different batches, alongside salt and seawater samples from Halen Môn. All samples were prepared to approximately 100 mg/L with respect to Na^+, and the dry salt samples were prepared as per NeilMed nasal rinse. In total 56 samples were tested. Calibration curves for Ca^{2+} (A), Mg^{2+} (B), K^+ (C) and Na^+ (D) can be seen in Appendix D.

A multi-ion standard was prepared from certified reference material TraceCERT®, 1000 mg/L Na^+ in water, 1000 mg/L K^+ in water, 1000 mg/L Ca^{2+} in nitric acid and Mg^{2+} in nitric acid. Calibration standards were prepared in triplicate, concentrations can be seen in Table 65.

Table 65 - Calibration concentrations using individual standards in mg/L for Nasal Spray 10 product Optimised Bulk Metal Analysis

Ion	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7
Na^+	5	25	50	75	100	150	
K^+	0.375	1.875	3.75	5.625	7.5	11.25	15
Mg^{2+}	1.25	6.25	12.5	18.75	25	37.5	50
Ca^{2+}	1.25	6.25	12.5	18.75	25	37.5	50

3.9 **ICP-OES**

All chemicals were purchased from Fisher Scientific or Sigma Aldrich and used as directed. All analysis was carried out using an Agilent Varient ICP-OES Machine.

3.9.1 **ICP-OES Full Cation Semi-Qualitative Analysis.**

Dilute samples of the 10 products (concentration approx. 5 mg/L with respect to Na^+) were prepared; a total of 30 samples including replicates. Replicate HPLC water samples were also analysed. HPLC water was used in the dilution of the samples.

3.9.2 **ICP-OES Full Cation Quantitative Analysis.**

A multi-ion standard was prepared from TraceCERT®, 1000 mg/L Na^+ in HNO_3 (1M), 1000 mg/L K^+ in HNO_3 (1M), 1000 mg/L Mg^{2+} in HNO_3 (1M), 1000 mg/L Ca^{2+} in HNO_3 (1M), 1000 mg/L Cu^{2+} in HNO_3 (1M), 1000 mg/L Fe^{2+} in HNO_3 (1M), 1000 mg/L Zn^{2+} in HNO_3 (1M) and 1000 mg/L Mn^{2+} in HNO_3 (1M). Calibration standards were prepared as displayed in Table 66.

Table 66 – Calibration standards used in ICP-OES analysis 2

	Mg/L				µg/L			
	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cu^{2+}	Fe^{3+}	Zn^{2+}	Mn^{2+}
Blank								
Std 1	5	0.5	1.25	1.25	5	5	5	5
Std 2	10	1	2.5	2.5	10	10	10	10
Std 3	15	1.5	3.75	3.75	15	15	15	15
Std 4	25	2.5	6.25	6.25	25	25	25	25
Std 5	50	5	12.5	12.5	50	50	50	50
Std 6	75	7.5	18.75	18.75	75	75	75	75
Std 7	100	10	25	25	100	100	100	100
Std 8	125	12.5	31.25	31.25	125	125	125	125

Sample solutions were prepared from the 10 commercial products, 2 batches in duplicate, and from 0.1 micron filtered and fully filtered Halen Môn seawater in triplicate. A total of 46 samples were analysed. The concentrations were determined using wavelengths of 422.673 nm for Ca^{2+}, 769.897 nm for K^{+}, 285.214 nm for Mg^{2+} and 588.995 nm for Na^{+}. These wavelengths gave the best linear calibration.

3.10 **AAS Bulk Metal Analysis of 6 Commercial Seawater Products**

All chemicals were purchased from Fisher Scientific or Sigma Aldrich and used as directed. All analysis was carried out using a Varian Spectra 220 FS AAS Machine.

Calibration standards were prepared from TraceCERT®, 1000 mg/L Na^{+} in HNO_3 (1M), 1000 mg/L K^{+} in HNO_3 (1M), 1000 mg/L Mg^{2+} in HNO_3 (1M), 1000 mg/L Ca^{2+} in HNO_3. Dilution was with ultra-pure water. The calibration standards used can be seen in Table 67.

Table 67 - Calibration standards used in AAS analysis 1 for Na^{+}, K^{+}, Ca^{2+} and Mg^{2+}

	Na^{+} mg/L	K^{+} mg/L	Ca^{2+} mg/L	Mg^{2+} mg/L
Blank	Blank	Blank	Blank	Blank
Standard 1	0.25	0.25	0.25	0.25
Standard 2	0.5	0.5	0.5	0.5
Standard 3	0.75	1	1	1
Standard 4	1	1.5	1.5	1.5
Standard 5	1.25	2	2	2
Standard 6	1.5			

The cations were analysed at the wavelengths seen in Table 68.

Table 68 - Wavelengths used to analyse product samples via AAS

	Na^{+}	K^{+}	Ca^{2+}	Mg^{2+}
Wavelength nm	589.6	766.5	422.7	202.6
Slit Size nm	1.00	1.00	0.50	1.00

Na^{+}, K^{+}, and Mg^{2+} were analysed in air and acetylene, with Ca^{2+} analysed using acetylene and nitrous oxide. Ten replicates of 6 products, 60 samples, ten replicate seawater samples and 10 ultra-pure water samples were analysed. During preparation, samples were serial diluted, using micro volumes of each product in 50 mL of ultra-pure water via micro pipette.

3.10.1 ICP-MS Trace Metal Analysis

All multi-ion standards were prepared by laboratory technicians at Aberystwyth University, with all analysis performed on an Agilent 7700 ICP-MS within the Department of Geography and Earth Sciences.

3.10.2 Initial Scoping Study of Trace Metal Content of Nasal Products using ICP-MS Analysis.

The standards contained more than 50 ions, only Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+} were of interest. The calibration standards can be seen in Table 69

Table 69 - Calibration standards used in ICP-MS initial scoping analysis of the seawater based nasal products

	Cu^{2+} ng/mL	Fe^{3+} ng/mL	Mn^{2+} ng/mL	Zn^{2+} ng/mL
Blank	Blank	Blank	Blank	Blank
Standard 1	10	10	10	10
Standard 2	20	20	20	20
Standard 3	50	50	50	50
Standard 4	100	100	100	100
Standard 5	200	200	200	200

Products were diluted to approximately 0.1% solution with respect to Na^{+} using ultra-pure water. Two replicates of each product, 6 seawater replicates, 5 deionised water and 5 ultra-pure water samples were analysed, giving 28 samples in total. All samples were 0.45 micron filtered prior to analysis. Fe^{3+} and Mn^{2+} were analysed with helium gas to mitigate interferences from argon gas. All analysis was performed using Microsoft Excel and IBM SPSS software.

3.10.3 Detailed Analysis of Trace Metal Content of Sea Water Based Nasal Products Analysed in section 3.6.1 using ICP-MS.

Multi-ion calibration standards were prepared by laboratory technicians at Aberystwyth University. The standards contained more than 50 ions, only Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+} were of interest. Calibration standards were the same as in Table 69. Products were diluted to approximately 0.1% solution with respect to Na^{+} using ultra-pure 10 replicates of each seawater-based product and 10 replicates of seawater were

analysed. During preparation, samples were serially diluted, using micro volumes of each product in 50 mL of ultra-pure water via micro pipette. All samples were 0.45 micron filtered prior to analysis. Fe^{3+} and Mn^{2+} were analysed with helium gas to mitigate interferences from argon gas. All analysis was performed using Microsoft Excel and IBM SPSS software.

4 Nasal Mucus Sample Collections

4.1 Mucus Collection

There was a requirement to find a less intrusive method with minimal pain or discomfort[77] for nasal mucus collection that will generate in excess of 200 mg for analysis. An earlier pilot study carried out at Bangor University (Appendix A), trialled two sample collection methods, one using a cotton wool plug, between 130 and 300 mg in size, and the other using an inert plastic splint, manufactured by Exmoor Plastics in each nostril. Both the cotton wool and the splint were inserted into the nasal cavity for 15 min prior to removal. The plastic splint proved problematic. The nasal mucus sample did not adhere to the splint making collection on removal an issue and was also found to be painful to the participants. The cotton wool method provided yields in the range of 110 – 1520 mg, compared to 80 – 460 mg using a splint (personal communication – Mr David Hill).

A decision was made to extend the study using commercially purchased cotton wool balls, Asda Little Angels™ manufactured from 100% cotton.

Cotton wool, a cellulose polymer, has hydrophilic properties, and liquids are absorbed through capillary action, absorbing up to 27 times its own weight.[78]

Ethics approval (IRAS154619) was obtained in conjunction with Betsi Cadwaladr Health Board to recruit participants for the study. The forms can be seen in Appendix B. Participants were recruited from healthy Bangor University students, and staff members working at Ysbyty Gwynedd in Bangor. Participants were considered healthy if they were not being treated for any nasal condition or outwardly displaying symptoms of the cold virus. A visual inspection of the nasal cavity was carried out by Mr David Hill, ENT consultant at Ysbyty Gwynedd, Bangor prior to collections taking place.

4.2 Results and discussion, Healthy Volunteers

The masses of cotton wool used in these collections had an average mass of 0.13 g.

Participants were given the option to terminate the procedure if it caused pain or distress. No participants withdrew from the study. There was a wide range in the mass

of mucus collected from 0.10 g to 1.07 g, with a mean of 0.34 g with a standard deviation of 0.17 g. Collections were similar for each nostril and can be seen in Table 70. The one-way ANOVA in Table 71 shows that the values for the left and right nostril are statistically similar.

Table 70 - Mean values for mucus collected from healthy volunteers with a left right comparison, compiled using SPSS

Descriptives								
Mucus Mass (g)								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Left	40	0.33	0.15	0.02	0.28	0.38	0.14	0.74
Right	40	0.35	0.19	0.03	0.29	0.41	0.1	1.07
Total	80	0.34	0.17	0.02	0.30	0.38	0.1	1.07

Table 71 - One -way ANOVA for nasal mucus showing statistically similar results, compiled using SPSS

ANOVA					
Mucus Mass (g) Left/Right					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.008	1	0.008	0.292	0.591
Within Groups	2.247	78	0.029		
Total	2.256	79			

The collection means were also compared for male and female volunteers. These can be seen in Table 72 with the one-way ANOVA showing that the means for male and female were statistically similar in Table 73.

Table 72 - Mean values for mucus collected from healthy volunteers with a male female comparison, compiled using SPSS

Descriptives								
Mucus Mass (g)								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Male	40	0.35	0.19	0.03	0.29	0.41	0.12	1.07
Female	40	0.33	0.15	0.02	0.28	0.38	0.1	0.75
Total	80	0.34	0.17	0.02	0.30	0.38	0.1	1.07

Table 73 - One -way ANOVA for nasal mucus showing statistically similar results for male and female volunteers compiled using SPSS

ANOVA					
Mucus Mass (g)					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.006	1	0.006	0.2	0.656
Within Groups	2.25	78	0.029		
Total	2.256	79			

The histogram in Figure 47 displays the frequency of mass collected. The Pearson correlation coefficient[79] was calculated to see if there was a correlation between the mass of the cotton wool and the mass of mucus collected. A value of +1 would indicate a positive correlation, -1 a negative correlation and a value close to 0 indicates there is no correlation. The results gave a coefficient of -0.0663, indicating no correlation between the mass of cotton wool and the mass of mucus collected.

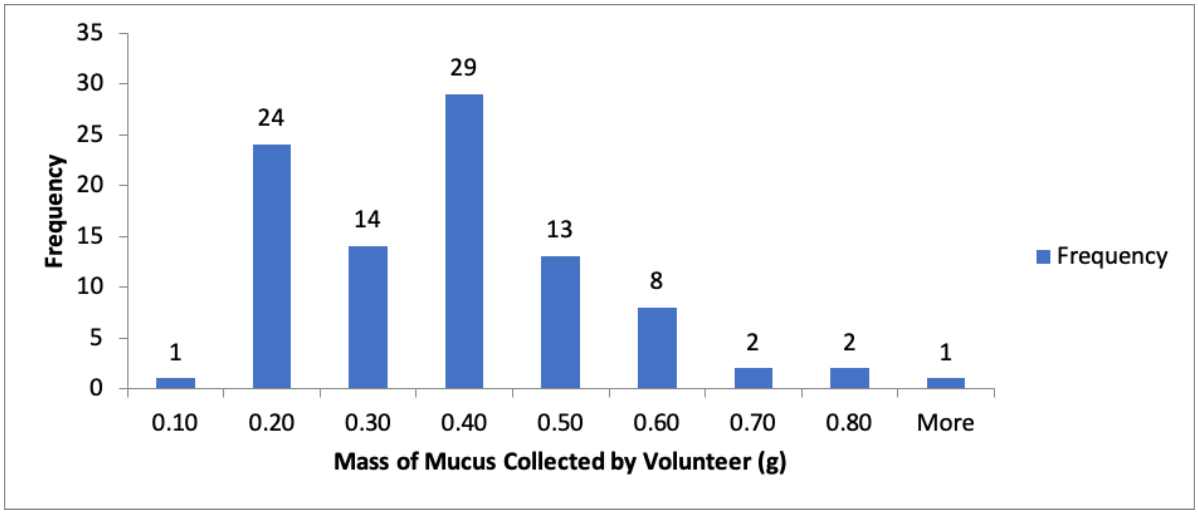


Figure 47 - Histogram showing mass of mucus collected from healthy volunteers

The scatter graph in Figure 48 displays the relationship.

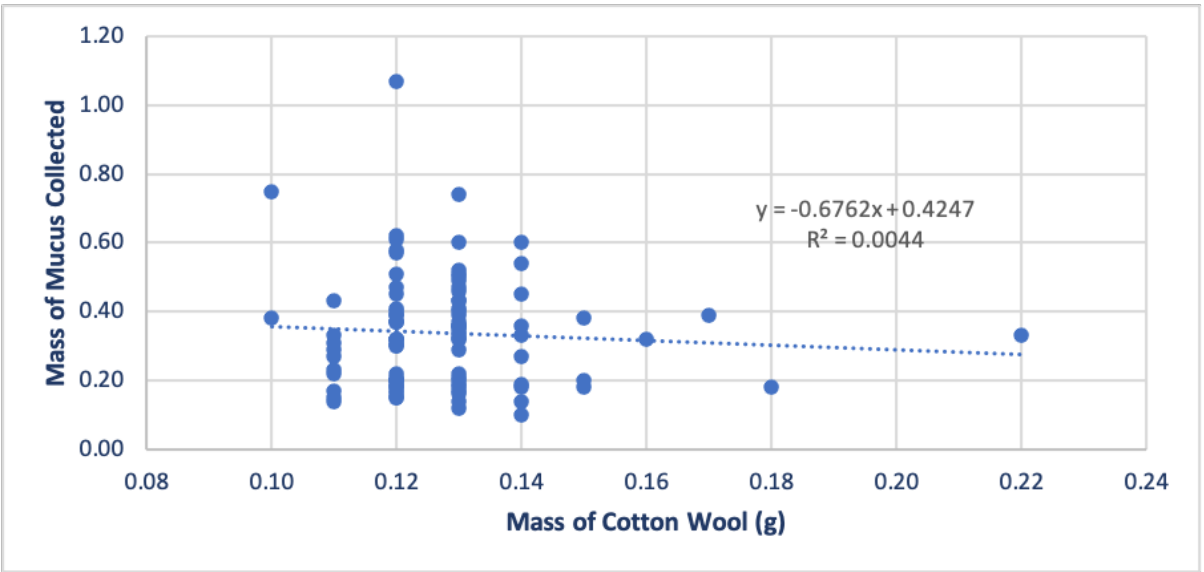


Figure 48 – Scatter graph depicting the relationship between the mass of cotton wool and the mass of nasal mucus collected

The experience of the participants was of great importance. Each participant was asked to rate their pain using visual analogue scale (VAS),[80] as seen in Figure 49.

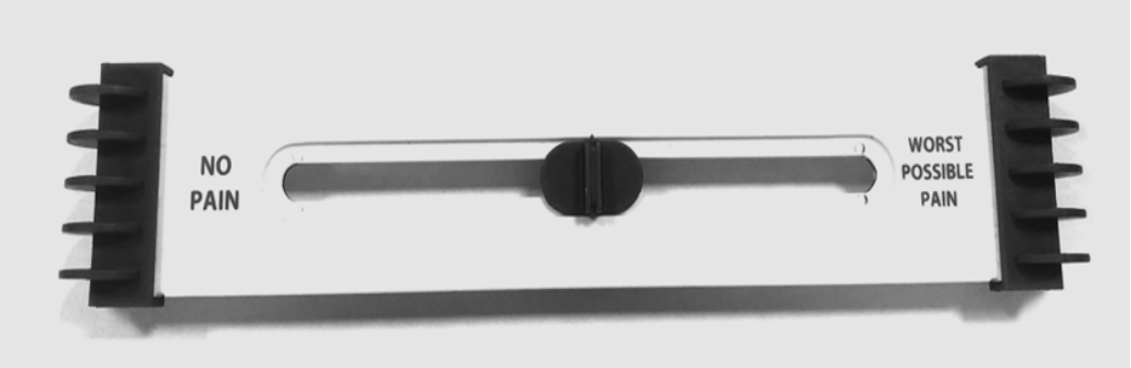


Figure 49 - Visual Analogue Scale (VAS)

The average VAS pain score was 1.9, with most participants stating the procedure was more uncomfortable than painful. Figure 50 displays the pain scores by frequency.

Out of those that expressed a score over 5, only 1 would not recommend the procedure to a friend and would not do it again. 2 of those who expressed a high pain score, had stated that insertion of the cotton wool had not caused any pain. Participants were also asked to give 3 words to describe their experience.

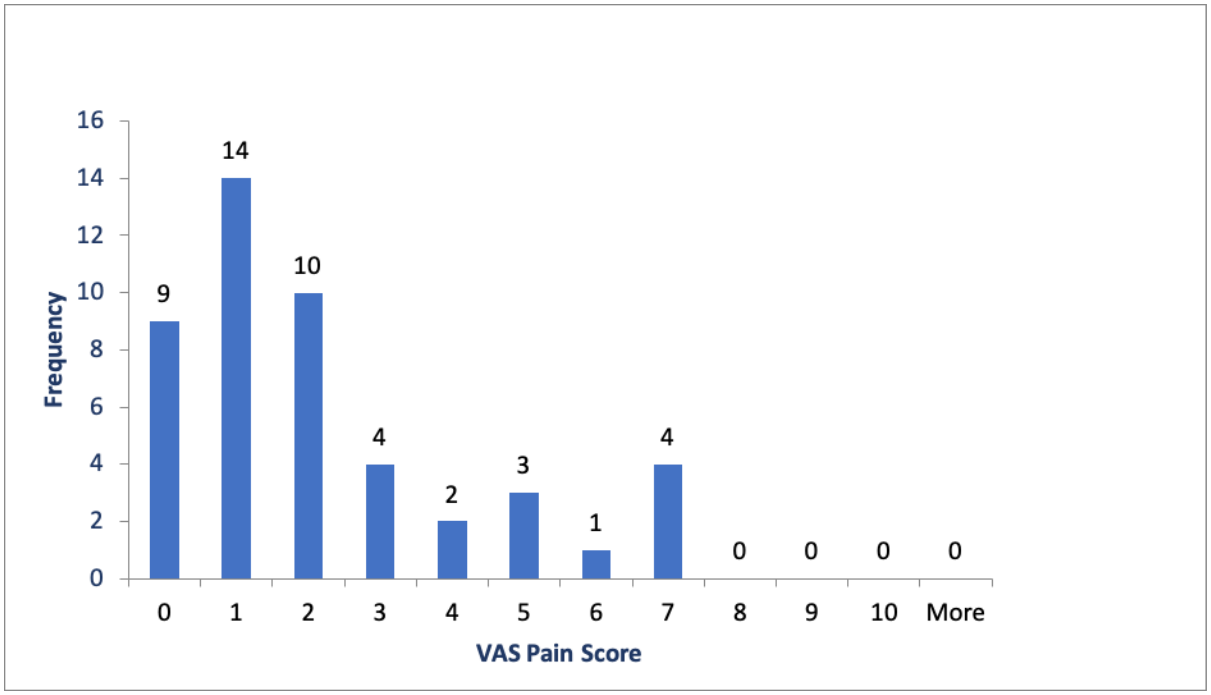


Figure 50 - Histogram depicting pain scores during mucus collection

A word cloud was generated from these words using Microsoft WordCloud as seen in Figure 51. This has been generated based on the frequeny of words given by the volunteers. The process was described as painless, weird, odd, surprising and strange. The majority of participants did not report pain,[81] but slight discomfort on insertion of the cotton wool but would not be averse to participating again in the future. There was no correlation between the mass of the cotton wool and the VAS pain scores. Participants with larger pieces of cotton wool reported low VAS scores.

This method of nasal mucus collection gave reproducible results and was acceptable to all participants.

Figure 51 - Words used to describe collection process generated in Microsoft Word

4.3 **Experimental**

4.3.1 **Cotton Wool Mucus Collections**

A consistent method to collect the mucus was used to collect all samples. The same brand of cotton wool, Asda Little Angels cotton wool balls, was used. Pieces of cotton wool were shaped into small splints or pellets approximately 4 cm in length by 1 cm in width with an accurate weighted average mass of 0.12 g as seen in Figure 52.

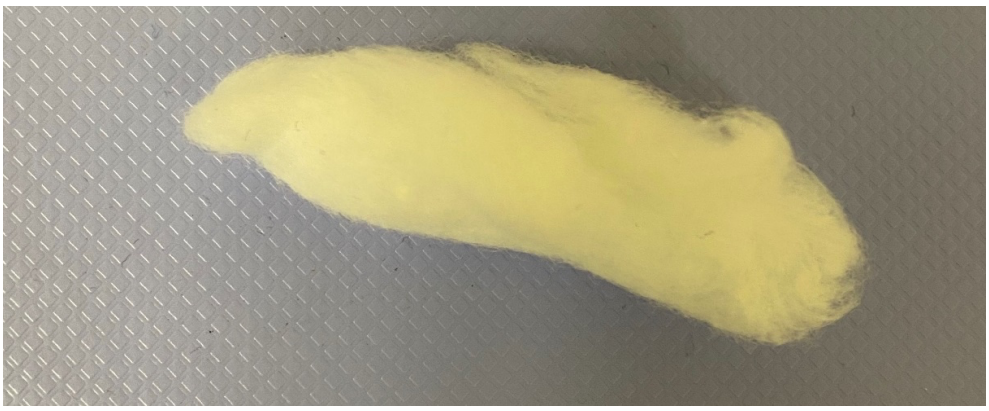


Figure 52 - Cotton wool splint used to collect mucus

The prepared splints were placed into pre-weighed, labelled sample tubes, 50 mL screw topped centrifuge tubes seen in Figure 53.

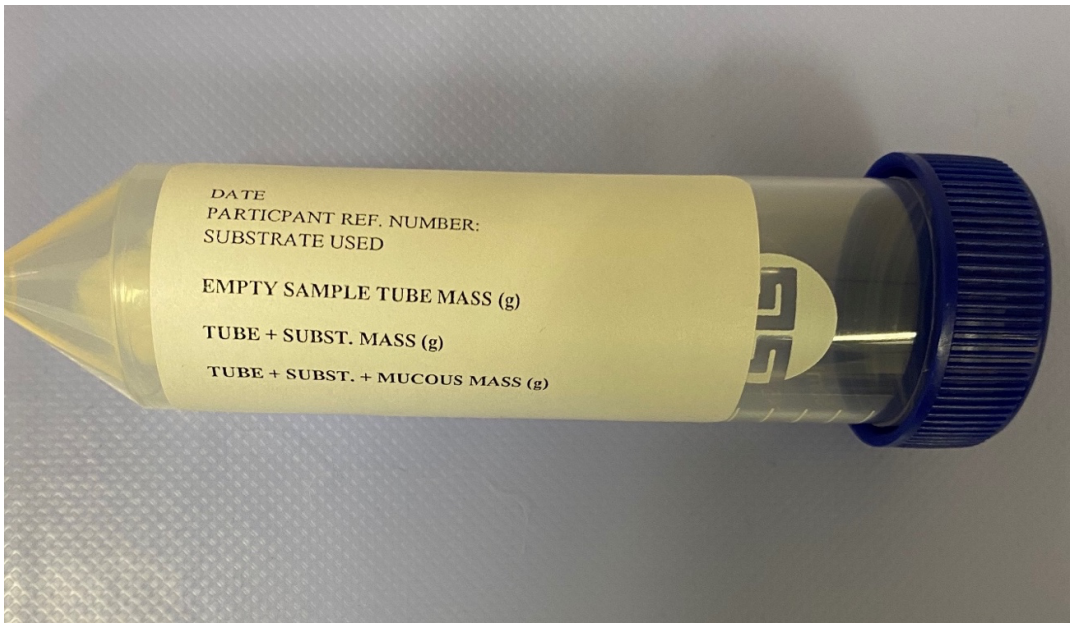


Figure 53 - Sample collection tube

These were prepared in advance of collection. On the collection date a pair of tubes were assigned to a recipient, one for the left nostril and one for the right nostril. The collection process took 15 min and the cotton wool containing the mucus was returned to the sample vial, re-weighed and the mass of mucus collected noted. The samples were then stored at -22 °C until the samples were thawed for analysis. Figure 54 shows the sample collection process.

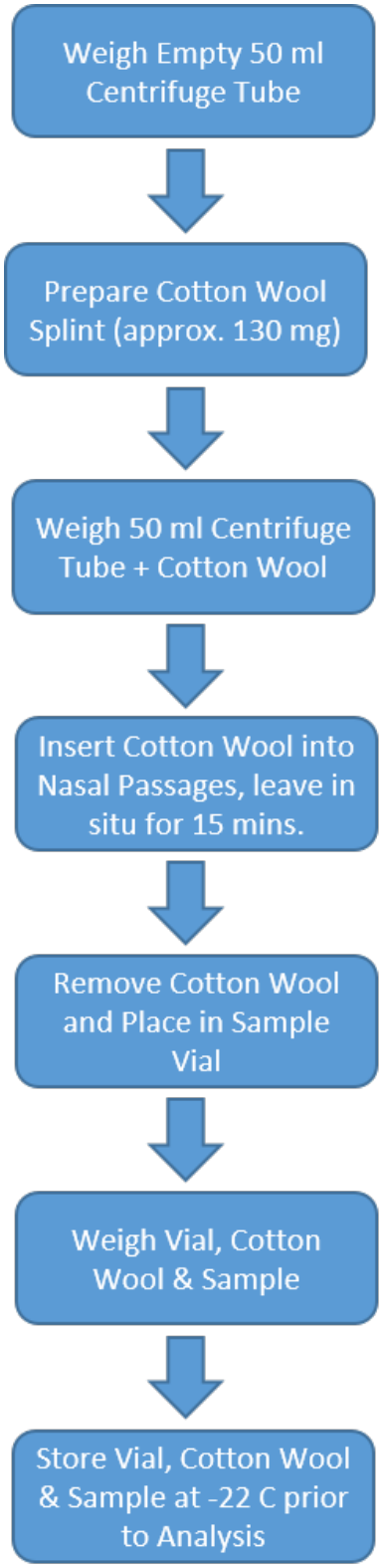


Figure 54 - Nasal mucus sample collection process

4.3.2 **Nasal Mucus Collection, Healthy Volunteers**

Participants were recruited from healthy volunteers at Bangor University and Ysbyty Gwynedd in Bangor. 47 participants in total were recruited, 40 Bangor University students, 20 male and 20 females, with 7 staff members (gender not recorded) from Ysbyty Gwynedd in Bangor. Sample collection vials were prepared following the process in section 4.3.1, two sample tubes per participant for left and right nasal passages.

Participants were asked brief questions regarding their general health and a brief nasal examination and asked to read the information sheet regarding analysis of nasal mucus in healthy adults (168), before signing the consent form (168). Mr David Hill, ENT Consultant at Ysbyty Gwynedd inserted a cotton wool plug into the right nostril followed by another cotton wool plug in the left. The participants remained seated in a warm room for 15 min. On removal, the cotton wool plug containing the mucus sample were removed, placed in pre-weighed 50 mL screw topped sample vial and re-weighed. Participants were then asked to complete a brief questionnaire regarding their experience (appendix D). Collected samples were taken to Ysbyty Gwynedd or storage at -22 °C to await analysis.

Participants were asked to move the slider from the central position depending on the level of pain experienced. A value from 0 to 10 was recorded from the numbers on the reverse of the scale, corresponding to the location of the slider. The reverse of the scale can be seen in Figure 55.

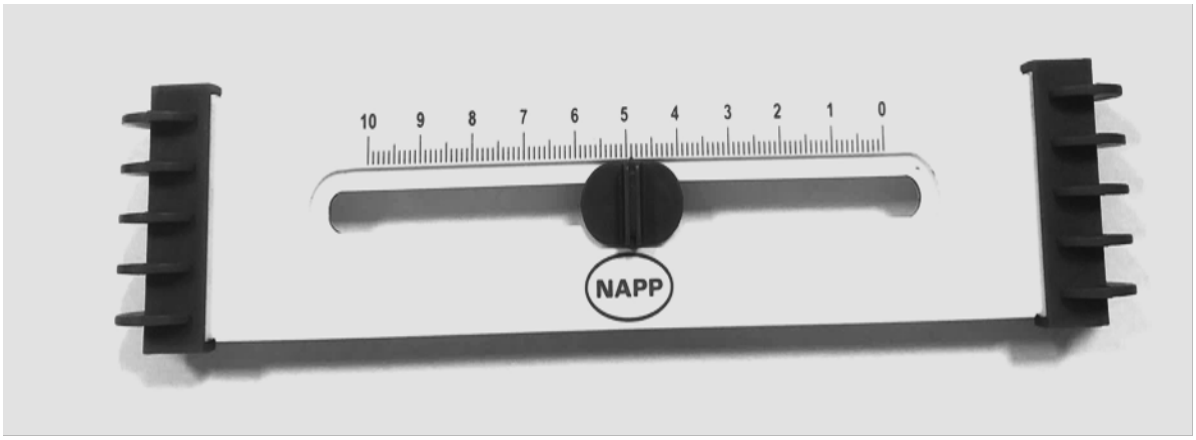


Figure 55 - Back of the Visual Analogue Scale (VAS)

5 Sample Digestion Method Development and Cotton Wool Analysis

A number of samples were collected from healthy participants. A small selection of these samples was selected to validate analytical methods. Sample ID's 120001 – 120014 were used for method development.

Cotton wool had been chosen as the collection matrix due to its absorbent nature.[78] in Section 4 Cotton wool was proven to be an acceptable mode of collection by previous participants and was easy to mould to ensure a fit in all nasal cavities.

The characteristics that make cotton wool a good matrix for collection, however, can cause complications when it comes to analysis. It is difficult to extract the mucus from the cotton wool. Due to the low concentration of trace metals in nasal mucus, only a small quantity of solvent can be used to solubilise the mucus. A method that extracts the maximum level of bulk and trace metals needed to be found.

Two solubility methods were selected: a mild method using ultra-pure water and a full digestion method using 68% nitric acid. The samples were then analysed using inductively coupled plasma mass spectrometry (ICP-MS), ion chromatography (IC) and atomic absorption spectroscopy (AAS). The data obtained from each was used to determine which method of solubilisation and mode of analysis would be used moving forward. The results gave indicative values for trace and bulk metals in nasal mucus samples and the cotton wool pellets.

Sample vials containing cotton wool with 5 or 10 mL ultra-pure water or 2 mL nitric acid were prepared and analysed alongside the nasal mucus samples to understand the bulk and trace metal contribution from the cotton wool.

Control samples of cotton wool (of approximately the same mass used in mucus collections), and mucus samples were treated using the method seen in Figure 62. Samples were analysed using ICP-MS, IC and AAS where applicable.

The second method was a complete digestion of the cotton wool containing the mucus sample using 68% nitric acid. Nasal mucus samples were suspended on cotton wool

of approximately 130 mg in mass. Both the mucus and cotton wool control samples followed the process flow outlined in Figure 63.

5.1 Results and Discussion

5.1.1 ICP-MS Water Solubilisation

ICP-MS analysis was performed using an Agilent 7700 ICP-MS at Aberystwyth University for Al^{3+}, Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+}.

Calibration standards were prepared by their laboratory technician. The ICP-MS was calibrated from 0 – 200 ng/mL for 50+ elements and trace metals with a Ru standard. Calibration curves for the 5 trace metals of interest can be seen in Appendix I.

The calibration data can be seen in Table 74.

Table 74 - Calibration data ICP-MS water solubilisation analysis

			27 Al [tune 1]		27 Al [He mode]		55 Mn [He mode]		56 Fe [He mode]		63 Cu [tune 1]		66 Zn [tune 1]	
Type	Level	Sample Name	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD
CalBlk	1	Blk	0.000	N/A	0.000	N/A	0.000	N/A	0.000	N/A	0.000	N/A	0.000	N/A
CalStd	3	10 ng/mL	14.377	0.894	13.460	3.496	9.652	2.348	6.669	6.537	9.913	1.220	6.812	0.620
CalStd	4	20 ng/ml	24.648	0.775	24.285	1.719	20.390	1.155	18.079	1.373	22.688	0.783	19.943	2.434
CalStd	5	50 ng/mL	52.597	0.926	52.370	5.137	51.462	3.777	51.587	3.395	54.888	1.760	52.752	2.845
CalStd	6	100 ng/mL	100.448	2.205	95.365	1.476	94.936	1.975	97.295	3.649	102.198	1.546	101.640	4.192
CalStd	7	200 ng/mL	198.443	4.040	201.124	1.381	202.145	0.704	201.314	1.679	197.414	0.021	198.657	0.512

The limit of detection and limit of quantification for the 5 trace elements can be seen in Table 75.

Table 75 - LOD and LOQ for Al^{3+}, Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+}- ICP-MS water solubilisation

	LoD (ng/ml)	LoQ (ng/ml)
Al^{3+}	0.069	0.243
Cu^{2+}	0.710	6.106
Fe^{3+}	0.865	2.215
Mn^{2+}	0.053	0.061
Zn^{2+}	1.393	3.582

5.1.2 Trace Metal Analysis of Cotton Wool Using Water Solubilisation Method

Cotton wool balls purchased from Asda were used in the collection of nasal mucus. Samples of the same batch of cotton wool were also analysed independently as it was unclear what level of trace metals may be present naturally in cotton wool. Control samples of cotton wool, with a similar mass to that of the mucus sample, approximately 0.13 g, were prepared and treated as outlined in Figure 62.

A small number of control samples were analysed. Four samples, two treated with 5 mL of ultra-pure water and two treated with 10 mL of ultra-pure water were prepared. With samples treated with 10 mL of ultra-pure water it was possible to obtain 2 replicates. The absorbance of the cotton wool made it problematic to extract the eluted sample from the matrix even after the sample had been centrifuged. Approx. 3 mL of elute was extracted from the control sample on adding 5 mL of water, and approx. 8 mL on adding 10 mL. The control samples analysed can be seen in Table 76.

Table 76 - Control samples treated with water used for ICP-MS

	Cotton Wool (g)	Water (ml)	Replicate
Control 1	0.13	5	a
Control 2	0.13	10	a
			b
Control 3	0.12	5	a
Control 4	0.12	10	a
			b

The raw data can be seen in Table 77. Helium gas was used instead of argon when analysing $Al^{3+,}$ Mn^{2+} and Fe^{3+} to increase sensitivity.

Table 77 - Control sample data ICP-MS water solubilisation analysis. The numbers in red indicate samples that have high relative standard deviations.

			27 Al [tune 1]		27 Al [He mode]		55 Mn [He mode]		56 Fe [He mode]		63 Cu [tune 1]		66 Zn [tune 1]	
Type	Level	Sample Name	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD
Sample		control 1	36.393	29.696	32.302	7.811	14.142	1.433	42.169	2.816	0.964	10.371	17.403	1.680
Sample		control 2a	21.784	0.968	31.184	11.701	7.021	3.119	31.778	21.079	0.424	0.571	8.403	2.215
Sample		control 2b	23.078	4.078	29.627	11.071	7.324	2.541	32.512	2.678	0.710	19.308	7.836	8.851
Sample		control 3	31.975	18.606	33.749	3.033	14.219	1.072	42.169	0.084	3.775	0.477	27.512	0.531
Sample		control 4a	43.219	46.816	34.956	4.832	7.952	0.441	50.736	24.920	0.589	3.023	8.014	0.815
Sample		control 4b	42.687	20.812	40.071	3.821	7.579	4.503	38.999	4.103	0.573	3.658	5.940	0.029

Metal concentrations across the 5 mL and 10 mL samples can be seen in Table 78 and Table 79 respectively.

Table 78 - ICP-MS results for 5 mL of added water to the cotton wool pellet

5 ml	Al^{3+} (ng/ml)	Mn^{2+} (ng/ml)	Fe^{3+} (ng/ml)	Cu^{2+} (ng/ml)	Zn^{2+} (ng/ml)
control 1	32.302	14.142	42.169	0.964	17.403
control 3	33.749	14.219	42.169	3.775	27.512
Mean	**33.025**	**14.181**	**42.169**	**2.370**	**22.458**

Table 79 - ICP-MS results for 10 mL of added water to the cotton wool pellet

10 ml	Al^{3+} (ng/ml)	Mn^{2+} (ng/ml)	Fe^{3+} (ng/ml)	Cu^{2+} (ng/ml)	Zn^{2+} (ng/ml)
control 2a	31.184	7.021	31.778	0.424	8.403
control 2b	29.627	7.324	32.512	0.710	7.836
control 4a	34.956	7.952	50.736	0.589	8.014
control 4b	40.071	7.579	38.999	0.573	5.940
Mean	**33.959**	**7.469**	**38.506**	**0.574**	**7.549**
SD	**4.648**	**0.395**	**8.775**	**0.117**	**1.098**

The concentrations for Al^{3+} were similar in both the 5 mL and 10 mL added water samples, as was Fe^{3+}. If all the natural cations in the cotton wool were solubilising it would be expected that the 5 mL control sample would be twice the value if the 10 mL control sample. The Mn^{2+} concentration in the 10 mL sample was approximately 50% of the 5 mL sample and the Zn^{2+} concentration in the 10 mL was approximately 33% of that in the 5 mL sample. The Cu^{2+} concentrations varied considerably between the 5 mL control samples. The concentrations were similar within the 10 mL sample. Mean values were used to give an indicative value for the metal content contribution from the cotton wool when analysing the nasal mucus samples.

5.1.3 Trace Metal Analysis of Nasal Mucus Using Water Solubilisation Method

Sample ID's 120001 to 120008 from 4 participants, 8 samples, were selected to assess the viability of using ultra-pure water to solubilise the nasal mucus suspended upon the cotton wool. Water was added to the samples, 5 mL to the odd numbered samples, right nostril, and 10 mL to the even numbered samples, left nostril. On adding

water to the mucus samples the samples in Table 80 were produced. Where 10 mL of water was used in the solubilisation 2 replicates a and b were analysed.

Table 80 - Small sample set used in mucus analysis via ultra-pure water

		Cotton Wool (g)	Mucus (g)	Water (ml)	Replicate
120001	R	0.18	0.18	5	a
120002	L	0.15	0.18	10	a
120003	R	0.14	0.45	5	a
120004	L	0.12	0.37	10	a
					b
120005	R	0.17	0.39	5	a
120006	L	0.14	0.19	10	a
					b
120007	R	0.14	0.6	5	a
120008	L	0.13	0.49	10	a
					b

The samples treated with 5 mL of water remained highly viscous. This made obtaining a viable sample after filtration problematic. Samples of ultra-pure water were also analysed. Where extraction allowed, 2 replicates were produced and analysed.

In total 11 samples were analysed via ICP-MS. These can be seen in Table 81. The ICP-MS was run in helium mode to analyse $Al^{3+,}$ Mn^{2+} and Fe^{3+} to eliminate interference and increase sensitivity.[82]

Table 81 – ICP-MS raw data for nasal mucus analysis samples 120001 – 120008) with %RSD. Sample ID 120001 did not contain sufficient volume to obtain a reading

		27 Al [He mode]		55 Mn [He mode]		56 Fe [He mode]		63 Cu [tune 1]		66 Zn [tune 1]	
Side of Nose	Sample Name	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD
R	120001	5.8757	126.6759	0.2723	146.6682	<0.000	N/A	1.7094	130.0756	1.6216	370.2989
L	120002	99.5326	1.9619	8.3068	1.4939	32.7488	2.5535	13.6085	0.1680	43.4871	0.5519
R	120003	115.5890	1.9194	11.1533	1.0974	23.4468	1.2075	14.6308	1.8613	60.2959	0.6191
L	120004a	22.4821	4.6766	4.7976	1.2752	8.3537	0.5457	7.5561	0.0848	12.5584	0.3261
L	120004b	18.1870	9.3272	5.2234	1.6322	8.4369	2.1790	7.5022	1.5581	17.1277	1.3890
R	120005	78.3178	1.1670	3.7899	1.0759	14.1550	1.4208	3.3940	0.2269	20.5120	2.7936
L	120006a	91.1810	0.9786	6.7261	1.6369	65.7749	5.1811	18.9004	0.4993	52.2181	0.2507
L	120006b	79.0040	1.8483	6.3230	2.8148	64.3195	3.4925	10.6171	0.6318	29.7115	0.0960
R	120007	102.9974	2.9674	9.5115	0.4150	56.7596	0.9609	37.5540	2.0422	61.8748	1.6286
L	120008a	89.6654	1.1431	5.3466	1.9887	22.6191	0.0133	19.9393	0.1722	38.3758	1.1804
L	120008b	59.1401	2.6457	5.4815	2.1376	19.5797	1.6155	23.1048	6.7408	32.5812	4.9532

The odd numbered samples (right hand nostril) were treated with 5 mL of water. Sample 120001 was extremely viscous and on solubilisation only produced 1.5 mL of

solution. This sample, on analysis, gave high %RSD values for all metals and was not used in the analysis.

The mass of the cotton wool used for the sampling of nasal mucus varied from 0.12 g to 0.18 g, with a mean of 0.13 g. From the results of section 5.1.2, cotton wool analysis using water, mean values (± standard deviation) were generated for Cu^{2+}, $Al^{3+,}$ Mn^{2+}, Zn^{2+} and Fe^{3+} concentrations. The standardised values of the metal ions in the cotton wool, enable the concentration in the mucus to be determined. The values for the cotton wool treated with 5 mL and 10 mL of water can be seen in Table 82 and Table 83 respectively.

Table 82 - Standardisation of cotton wool treated with 5 mL water

Mass (g)	Water (ml)	Cu^{2+} (ng/ml)	SD	Fe^{3+} (ng/ml)	SD	Zn^{2+} (ng/ml)	SD	Mn^{2+} (ng/ml)	SD	Al^{3+} (ng/ml)	SD
0.1	5	1.944	1.700	33.789	1.911	18.157	6.745	11.364	0.687	26.486	2.317
0.14	5	2.722	2.380	47.305	2.676	25.420	9.443	15.910	0.961	37.080	3.243
0.15	5	2.916	2.550	50.684	2.867	27.235	10.118	17.046	1.030	39.729	3.475
0.16	5	3.110	2.720	54.063	3.058	29.051	10.792	18.182	1.099	42.377	3.707
0.17	5	3.305	2.890	57.442	3.249	30.867	11.467	19.319	1.167	45.026	3.938

Table 83 - Standardisation of cotton wool treated with 10 mL water

Mass (g)	Water (ml)	Cu^{2+} (ng/ml)	SD	Fe^{3+} (ng/ml)	SD	Zn^{2+} (ng/ml)	SD	Mn^{2+} (ng/ml)	SD	Al^{3+} (ng/ml)	SD
0.1	10	0.405	0.094	31.058	8.333	6.030	0.769	5.994	0.573	27.325	4.891
0.11	10	0.446	0.103	34.164	9.167	6.633	0.846	6.594	0.631	30.058	5.380
0.12	10	0.487	0.113	37.270	10.000	7.236	0.923	7.193	0.688	32.790	5.870
0.13	10	0.527	0.122	40.376	10.833	7.839	1.000	7.793	0.745	35.523	6.359
0.14	10	0.568	0.132	43.481	11.667	8.442	1.077	8.392	0.803	38.255	6.848
0.15	10	0.608	0.141	46.587	12.500	9.045	1.154	8.992	0.860	40.988	7.337

The ion concentration for the mucus samples suspended on cotton wool (CW) can be seen in Table 84, alongside the standardised ion concentration for the equivalent mass of cotton wool used during volunteer mucus extraction. The results, using 5 mL of water for solubilisation, for Cu^{2+}, Al^{3} and Zn^{2+} show a greater concentration when compared to the metal ion content in the cotton wool control samples. It was not possible to determine a concentration for Fe^{3+} or Mn^{2+} from the samples, the values fell below the LOQ.

Table 84 - Metal concentration in nasal mucus samples 120003,120005 and120007 in ng/ml with corresponding control sample concentrations and standard deviation (5mL added water)

		Cotton Wool (g)	Mucus (g)	Water (ml)	Cu^{2+} (ng/ml)	CW	SD	Fe^{3+} (ng/ml)	CW	SD	Zn^{2+} (ng/ml)	CW	SD	Mn^{2+} (ng/ml)	CW	SD	Al^{3+} (ng/ml)	CW	SD
120003	R	0.14	0.45	5	14.631	2.722	2.380	23.447	47.305	2.676	60.296	25.420	9.443	11.153	15.910	0.961	115.589	37.080	3.243
120005	R	0.17	0.39	5	3.394	3.331	2.890	14.155	57.442	3.249	20.512	30.867	11.467	3.790	19.319	1.167	78.318	45.026	3.938
120007	R	0.14	0.60	5	37.554	2.722	2.380	56.760	47.305	2.676	61.875	25.420	9.443	9.512	15.910	0.961	102.997	37.080	3.243

Table 85 - *Metal concentration in nasal mucus samples 120002,120004, 120006 and120008 in ng/ml, corresponding control sample concentrations with standard deviation (10 mL added water)*

		Cotton Wool (g)	Mucus (g)	Water (ml)	Cu^{2+} (ng/ml)	CW	SD	Fe^{3+} (ng/ml)	CW	SD	Zn^{2+} (ng/ml)	CW	SD	Mn^{2+} (ng/ml)	CW	SD	Al^{3+} (ng/ml)	CW	SD
120002	L	0.15	0.18	10	13.608	0.608	0.141	32.749	46.587	12.5	43.487	9.045	1.154	8.307	8.992	0.86	99.533	40.988	7.337
120004	L	0.12	0.37	10	7.529	0.487	0.113	8.395	37.27	10	14.843	7.236	0.923	5.011	7.193	0.688	20.335	32.79	5.87
120006	L	0.14	0.19	10	14.759	0.568	0.132	65.047	43.481	11.667	40.965	8.442	1.077	6.525	8.392	0.803	85.093	38.255	6.848
120008	L	0.13	0.49	10	21.522	0.527	0.122	21.099	40.376	10.833	35.479	7.839	1.000	5.414	7.793	0.745	74.403	35.523	6.359

In most cases the concentration of Fe^{3+} in the control cotton wool samples were greater than the in the mucus sample and, in all samples, the Mn^{2+} concentration in the control sample was greater than in the mucus sample. This could be due to cations being retained by the moisture still held in the cotton wool or the mucus not solubilising in the water. Cotton wool is a natural product, and some pellets may also contain more minerals naturally than others.

The cation concentrations in samples 120003, 120005 and 120007, solubilised in 5 mL of water, less the value attributed to the cotton wool pellet, can be seen in Table 86 with the one-way ANOVA confirming that these values are significantly different in Table 87.

Table 86 - Cation concentration in samples 120003, 120005 and 120007, solubilised in 5mL of water less the value attributed to the cotton wool

	Cu^{2+} (ng/ml)	Fe^{3+} (ng/ml)	Mn^{2+} (ng/ml)	Zn^{2+} (ng/ml)	Al^{3+} (ng/ml)
120003	11.909	-23.858	-4.757	34.876	78.509
120005	0.064	-43.287	-15.529	-10.355	33.292
120007	34.832	9.455	-6.398	36.455	65.917

Table 87 - One-way ANOVA confirming the samples were statistically significantly different for samples 120003, 120005, 120007

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Cu	3	46.80	15.60	312.44		
Fe	3	-57.69	-19.23	711.48		
Mn	3	-26.68	-8.89	33.68		
Zn	3	60.98	20.33	706.58		
Al	3	177.72	59.24	544.60		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11147.53	4	2786.88	6.04	0.01	3.48
Within Groups	4617.56	10	461.76			
Total	15765.09	14				

The results for samples solubilised with 10 mL of water in Table 85.

The cation concentrations in samples 120002, 120004, 120006 and 120008, solubilised in 10 mL of water less the value attributed to the cotton wool pellet, can be seen in Table 88 with the one-way ANOVA showing that the values are statistically significantly different in Table 89.

Table 88 - Cation concentration in samples 120002, 120004, 120006 and 120008, solubilised in 10 mL of water less the value attributed to the cotton wool

	Cu^{2+} (ng/ml)	Fe^{3+} (ng/ml)	Mn^{2+} (ng/ml)	Zn^{2+} (ng/ml)	Al^{3+} (ng/ml)
120002	13.000	-13.838	-0.685	34.442	58.545
120004	7.042	-28.875	-2.182	7.607	-12.455
120006	14.191	21.566	-1.867	32.523	46.838
120008	20.995	-19.277	-2.379	27.64	38.88

Table 89 - One-way ANOVA confirming the samples were statistically significantly different for samples 120002, 120004, 120006 and 120007

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Cu	4	55.23	13.81	32.74		
Fe	4	-40.42	-10.11	484.48		
Mn	4	-7.11	-1.78	0.58		
Zn	4	102.21	25.55	151.34		
Al	4	131.81	32.95	981.59		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5217.66	4	1304.41	3.95	0.02	3.06
Within Groups	4952.15	15	330.14			
Total	10169.81	19				

As with the 5 mL samples, it was not possible to observe the concentration of Fe^{3+} or Mn^{2+} with the concentration in the control sample exceeding that in the mucus samples. This could be due to water being retained within the cotton wool. It was not possible to extract all eluent from the cotton wool. The mucus may not have solubilised in the water and may still be contained within the cotton wool. It was unclear if all ions were solubilised and extracted using this process,

5.1.4 **Results Water Solubilisation, IC**

The bulk metals in nasal mucus were too concentrated to be analysed by ICP-MS. IC was used to baseline the cation concentrations for Na^+, K^+, Mg^{2+} and Ca^{2+}.

Calibration data can be seen in Table 90 with the calibration curves in Appendix K

Table 90 – Calibration data table for solubilisation of cations in cotton wool and mucus using IC

Peak No.	Peak Name	Ret.Time min	Cal.Type	Eval.Type	Number of Points	Rel.Std. Dev %	Coeff.of Determination
1	Na^+	11.027	Lin	Area	13	2.2075	0.99911
2	K^+	18.317	Lin	Area	13	3.2233	0.99828
3	Mg^{2+}	20.59	Lin	Area	13	3.6358	0.9978
4	Ca^{2+}	27.66	Lin	Area	11	4.1788	0.99697

The limit of detection and limit of quantification can be seen in Table 91.

Table 91 - *LOD and LOQ for IC analysis*

	LoD mg/L	LoQ mg/L
Na^+	2.225	3.08
K^+	0.023	0.064
Mg^{2+}	0.065	0.141
Ca^{2+}	0.197	0.219

5.1.5 **Bulk Metal Analysis of Cotton Wool Using Water Solubilisation**

4 control samples, with cotton wool of a similar mass to that used in the mucus collections, were used for analysis. The cotton wool samples were prepared with ultra-pure water, as per the process in section 2.8, 2 with 5 mL of water and, 2 with 10 mL of water. These can be seen in Table 92.

Table 92 - Control Samples used in IC analysis (water)

	Cotton Wool (g)	Water (ml)
Control 1	0.13	5
Control 2	0.13	10
Control 3	0.12	5
Control 4	0.12	10

The raw data for the IC analysis can be seen in Table 93 for the cotton wool control samples that were solubilised with 5 mL of water and can be seen in Table 94 for those prepared in 10 mL of water.

Table 93 – Results IC Control sample mg/L in 5 mL water

5 ml	Na^+	K^+	Mg^{2+}	Ca^{2+}
C1	15.48	0.32	1.47	9.95
C1	15.97	0.32	1.31	9.95
C3	14.50	0.98	0.84	1.72
C3	14.72	0.96	0.86	1.72
Mean	15.17	0.65	1.12	5.83
SD	0.68	0.37	0.32	4.75

Table 94 - Results IC Control sample mg/L in 10 mL water

10 ml	Na^+	K^+	Mg^{2+}	Ca^{2+}
C2	8.05	0.09	0.38	0.98
C2	7.86	0.09	0.42	0.87
C4	8.44	0.08	0.41	0.79
C4	8.01	0.07	0.39	0.79
Mean	8.09	0.08	0.40	0.86
SD	0.25	0.01	0.02	0.09

All results were above the limit of quantification (Table 91). The mean results were used to approximate the cotton wool attribution in the ion concentration in the mucus and cotton wool samples solubilised with water. It is unclear if all trace metals were extracted using water for solubilisation. Nitric acid digestion of the cotton wool control samples and the cotton wool containing the mucus was also carried out for a comparison.

5.1.6 **Bulk Metal Analysis of Nasal Mucus Using Water Solubilisation**

IC was used to analyse the mucus samples for Na^+, K^+, Mg^{2+} and Ca^{2+}.

A small sample set consisting of sample ID's 120001 – 120008 from 4 participants, 8 samples, was selected to assess the viability of using ultra-pure water to solubilise the nasal mucus contained within the cotton wool. Water was added to the samples, 5 mL to the right nostril samples, and 10 mL to the left nostril samples. The samples were processed following the process in section 2.8. On adding water to the mucus samples the sample set seen in Table 95 was produced.

Table 95 – Samples prepared using water solubilisation for analysis via IC

		Cotton Wool (g)	Mucus (g)	Water (ml)	Replicate
120001	R	0.18	0.18	5	a
120002	L	0.15	0.18	10	a
120003	R	0.14	0.45	5	a
120004	L	0.12	0.37	10	a
					b
120005	R	0.17	0.39	5	a
120006	L	0.14	0.19	10	a
					b
120007	R	0.14	0.6	5	a
120008	L	0.13	0.49	10	a
					b

The samples treated with 5 mL of water remained highly viscous. This made obtaining a viable sample after filtration problematic. Control samples of ultra-pure water were also analysed.

Standardised values for the cotton wool element of the mucus sample were calculated for the added 5 mL and 10 mL of water. These can be seen in Table 96.

Table 96 - Cotton wool standardisation for added 10 mL (A) and added 5 mL (B) water

A	CW	10 ml	Na^+ mg/L	K^+ mg/L	Ca^{2+} mg/L	Mg^{2+} mg/L
	AVG	0.1	6.485	0.065	0.321	0.685
	SD	0.1	0.154	0.010	0.001	0.073
	AVG	0.11	7.133	0.072	0.353	0.754
	SD	0.11	0.169	0.011	0.001	0.080
	AVG	0.12	7.782	0.079	0.385	0.822
	SD	0.12	0.185	0.012	0.001	0.088
	AVG	0.13	8.430	0.085	0.417	0.891
	SD	0.13	0.200	0.013	0.001	0.095
	AVG	0.14	9.079	0.092	0.449	0.960
	SD	0.14	0.215	0.014	0.001	0.102
	AVG	0.15	9.727	0.098	0.482	1.028
	SD	0.15	0.231	0.015	0.002	0.110

B	CW	5 ml	Na^+ mg/L	K^+ mg/L	Ca^{2+} mg/L	Mg^{2+} mg/L
	AVG	0.1	12.14	0.53	0.89	4.54
	SD	0.1	0.63	0.37	0.30	4.66
	AVG	0.11	13.35	0.58	0.98	5.00
	SD	0.11	0.69	0.32	0.27	4.10
	AVG	0.12	14.56	0.63	1.07	5.45
	SD	0.12	0.75	0.44	0.36	5.59
	AVG	0.13	15.78	0.69	1.16	5.91
	SD	0.13	0.82	0.48	0.39	6.05
	AVG	0.14	16.99	0.74	1.24	6.36
	SD	0.14	0.88	0.51	0.42	6.52
	AVG	0.15	18.20	0.79	1.33	6.81
	SD	0.15	0.94	0.55	0.45	6.99
	AVG	0.17	20.63	0.90	1.51	7.72
	SD	0.17	1.07	0.62	0.52	7.92

The results taking the mass of cotton wool into account can be seen in Table 97 and pictorially in Figure 56.

Table 97 - Mucus results for solubilisation of Na, K, Mg and Ca in 5 mL and 10 mL added water for sample ID's 120001 - 120008

	Mass of CW (g)	Mass of Mucus (g)	Water mL	Na^+ in Sample mg/L	SD	K^+ in Sample mg/L	SD	Mg^{2+} in Sample mg/L	SD	Ca^{2+} in Sample mg/L	SD
120002	0.15	0.18	10.00	45.73	0.23	24.17	0.02	0.44	0.00	0.55	0.11
120003	0.14	0.45	5.00	224.12	0.88	66.02	0.51	-3.91	0.42	-1.46	6.52
120004	0.12	0.37	10.00	98.56	0.19	40.94	0.01	0.56	0.00	1.36	0.09
120005	0.17	0.39	5.00	-11.72	1.07	9.69	0.62	-7.66	0.52	-7.38	7.92
120006	0.14	0.19	10.00	52.65	0.22	28.46	0.01	0.36	0.00	0.96	0.10
120007	0.14	0.60	5.00	311.33	0.88	74.59	0.51	-3.79	0.42	-1.66	6.52
120008	0.13	0.49	10.00	139.99	0.20	47.42	0.01	0.52	0.00	1.37	0.10

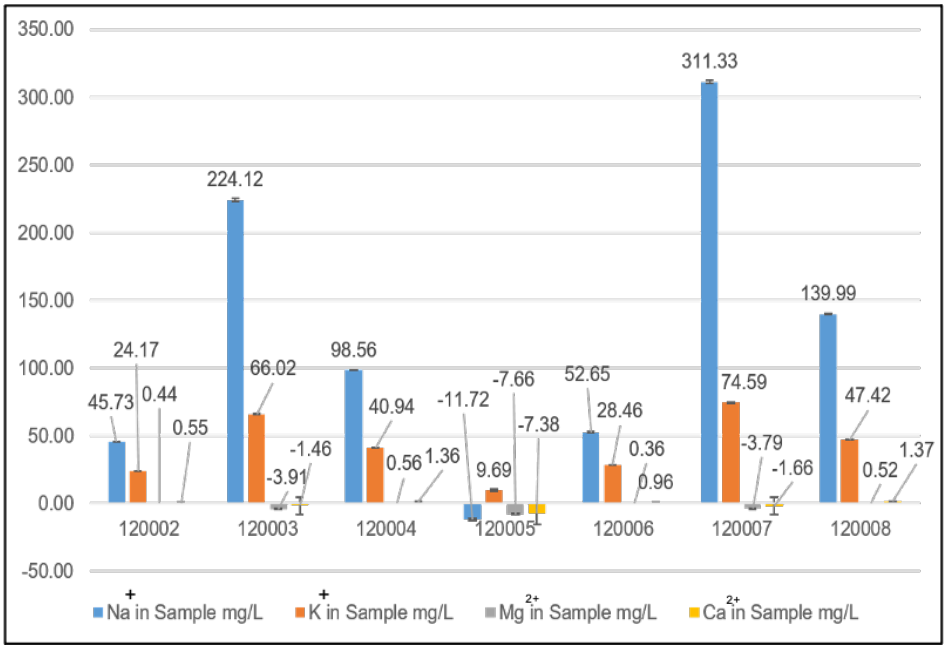


Figure 56 - Mucus results for solubilisation of Na^+, K^+, Mg^{2+} and Ca^{2+} in 5 mL and 10 mL added water, concentrations in mg/L

The results for solubilising mucus with water were varied and inconsistent. Using 5 mL of water gave a range from negative concentrations for Na^+ to 224 mg/L. It was also not possible to quantify concentrations for either Mg^{2+} or Ca^{2+}.

Using water does not give consistent results. Part of the nasal mucus sample may still be contained within the cotton wool pellet or suspended by the water absorbed by the cotton wool. The cotton wool is absorbing approximately 2 mL of any added water and it is unclear how much of the mucus sample is contained within this. Sample solubilisation with water will not be used moving forward.

5.2 ICP-MS, Nitric Acid Digestion

The ICP-MS was calibrated from 0 – 200 ng/mL for 50+ elements and trace metals using standards prepared by Aberystwyth University's departmental technician. Calibration curves for the 5 trace metals of interest can be seen in Appendix J.

The calibration data can be seen in Table 98.

Table 98 - *Calibration data ICP-MS analysis*

		27 Al [He mode]		55 Mn [He mode]		56 Fe [He mode]		63 Cu [tune 1]		66 Zn [tune 1]	
Level	Sample Name	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD
1	Blank	0.000	N/A	0.000	N/A	0.000	N/A	0.000	N/A	0.000	N/A
3	10 ng/mL	12.139	3.688	8.932	3.453	6.767	5.207	9.561	0.683	6.152	5.978
4	20 ng/ml	27.226	5.202	19.997	0.991	19.875	1.803	22.621	1.019	20.372	0.343
5	50 ng/mL	50.629	0.425	48.769	1.764	49.575	2.857	52.053	1.644	51.046	1.448
6	100 ng/mL	100.757	0.358	98.817	0.937	101.513	1.009	99.871	0.415	100.829	0.504
7	200 ng/mL	198.635	1.013	200.953	0.312	199.524	1.492	199.311	0.425	199.479	1.302

The limit of detection (LOD) and limit of quantification (LOQ) for the trace metals can be seen in Table 99.

Table 99 - LOD & LOQ for trace metals ICP-MS

	LoD (ng/ml)	LoQ (ng/ml)
Cu^{2+}	0.230	1.228
Fe^{3+}	0.151	8.188
Al^{3+}	1.132	2.668
Mn^{2+}	0.059	0.145
Zn^{2+}	0.447	5.507

5.2.1 Trace Metal Analysis of Cotton Wool Using Complete Nitric Acid Digestion Samples 120009 to 120014

The trace metal content for the digested cotton wool sample, with mean and standard deviation values can be seen in Table 100. The values in red are outliers. The outliers were determined using the interquartile rule, where IQR = Quartile 3 – Quartile 1. The IQR is multiplied by a constant (1.5). This value is added to Quartile 3 and subtracted from Quartile 1. Any values outside of these values would be considered an outlier.

Table 100 - Trace metal content in cotton wool (ng/ml) after nitric acid digestion with mean, SD and outliers, the red values indicate anomalous results.

Sample	Asda CW	Weight (g)	Nitric Acid (mL)	Cu^{2+}	Al^{3+}	Mn^{2+}	Fe^{3+}	Zn2+
1	A	0.1306	2	15.096	3673.523	24.130	224.957	22.109
2	B	0.1305	2	11.011	196.068	22.661	242.911	24.827
3	C	0.1308	2	17.301	233.080	23.224	354.412	32.492
4	D	0.1300	2	16.560	240.075	23.710	1563.906	44.304
5	E	0.1307	2	6.748	238.880	20.992	224.126	35.348
6	F	0.1303	2	7.882	185.871	22.318	216.261	45.235
7	G	0.1304	2	15.222	233.764	21.220	219.576	38.744
8	H	0.1303	2	10.190	232.751	22.145	427.186	34.049
9	J	0.1307	2	6.006	187.274	20.029	208.016	54.129
10	K	0.1303	2	6.665	254.741	23.154	240.852	21.304
Mean		0.1305		11.268	222.500	22.358	254.902	35.254
SD		0.0002		4.431	25.597	1.294	12.693	10.725

All values are above the limit of quantification in Table 99.

The nitric acid digestion method produced greater concentration levels for all trace elements when compared to water solubilisation. This can be seen in Figure 57.

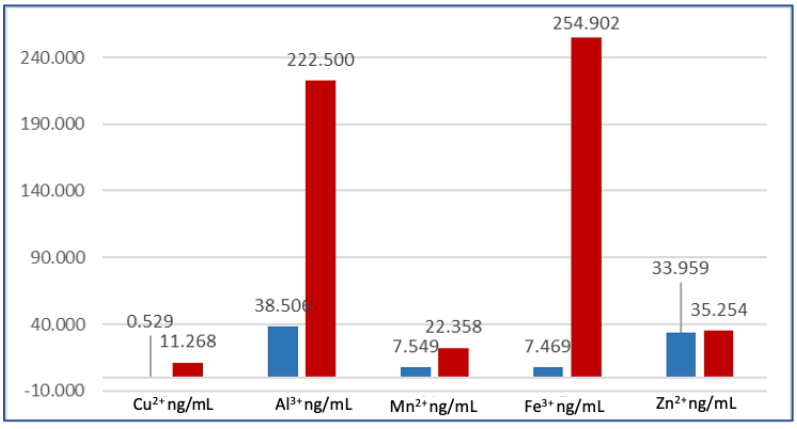


Figure 57 - Trace metal comparison between cotton wool treated in water (blue) and nitric acid (red) – all concentrations in ng/ml

This shows that by using water, only a small amount of the trace metals in the cotton wool was being solubilised, full digestion of the cotton wool and samples was used moving forward.

5.2.2 Trace Metal Analysis of Nasal Mucus Using Complete Nitric Acid Digestion

The details of the samples and the raw results for the digested cotton wool and mucus sample can be seen in Table 101.

Table 101 – ICP-MS raw results for nasal mucus samples 120009 - 120014 on cotton wool digested in nitric acid, all concentrations in ng/mL

Sample	CW Mass (g)	Nitric Acid (mL)	MUCUS (g)	Cu^{2+} ng/mL	Al^{3+} ng/mL	Mn^{2+} ng/mL	Fe^{3+} ng/mL	Zn^{2+} ng/mL
120009	0.1400	2	0.36	24.873	317.373	26.200	350.456	124.465
120010	0.1300	2	0.37	19.504	364.953	27.022	340.690	79.483
120011	0.1300	2	0.36	11.159	270.793	21.482	242.452	46.710
120012	0.1600	2	0.32	16.258	397.201	29.518	351.733	75.595
120013	0.1200	2	0.19	20.883	239.513	23.464	334.092	49.454
120014	0.1200	2	0.18	19.870	273.900	22.291	333.602	49.950

Standardised metal concentration values were calculated for trace metals for the contribution attributed by the cotton wool pellets. Values were calculated for the different sized pieces of cotton wool that were used in the mucus collection process. These can be seen in Table 102 and give a relative value for the metal contribution from the cotton wool in the sample.

Table 102 - Standardisation values for the cotton wool pellets digested in 68% nitric acid

CW	Cu^{2+} ng/mL	SD	Al^{3+} ng/mL	SD	Mn^{2+} ng/mL	SD	Fe^{3+} ng/mL	SD	Zn^{2+} ng/mL	SD
0.12	10.361	4.074	204.598	23.538	20.559	0.903	240.950	69.917	32.418	9.862
0.13	11.225	4.414	221.648	25.499	22.273	1.199	261.029	75.744	35.119	10.684
0.14	12.088	4.753	238.698	27.461	23.986	1.291	281.108	81.570	37.821	11.505
0.15	12.952	5.093	255.748	29.422	25.699	1.383	301.188	87.397	40.522	12.327
0.16	13.815	5.433	272.798	31.384	27.412	1.476	321.267	93.223	43.224	13.149

The metal concentration in the mucus samples in ng/mL and µmol/mL can be seen in Table 103. This can be seen pictorially for µmol/mL in Figure 58 and in ng/mL in Figure 59.

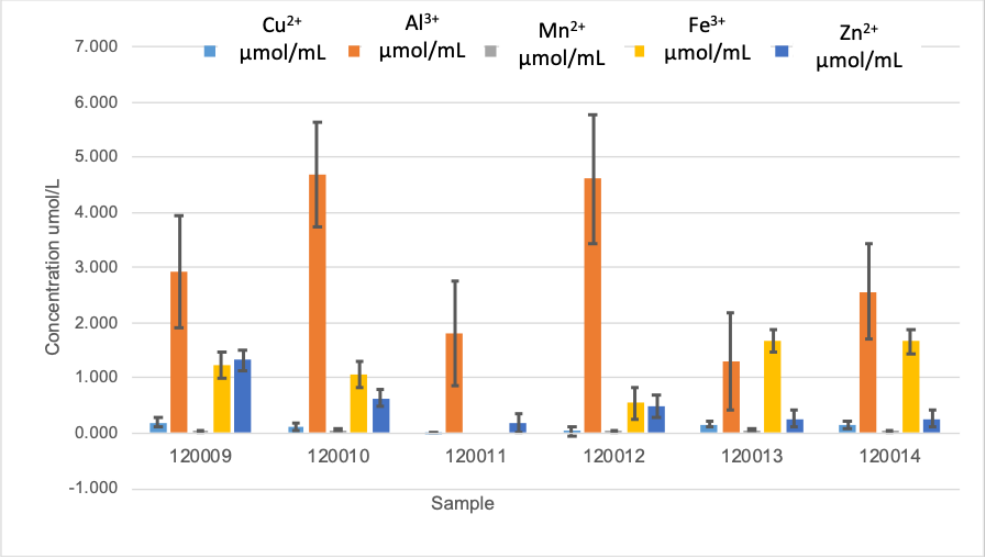


Figure 58 - ICP-MS concentrations for Cu^{2+}, Al^{3+}, Mn^{2+}, Fe^{2+} and Zn^{2+} in ng/mL in samples 120009 - 120014

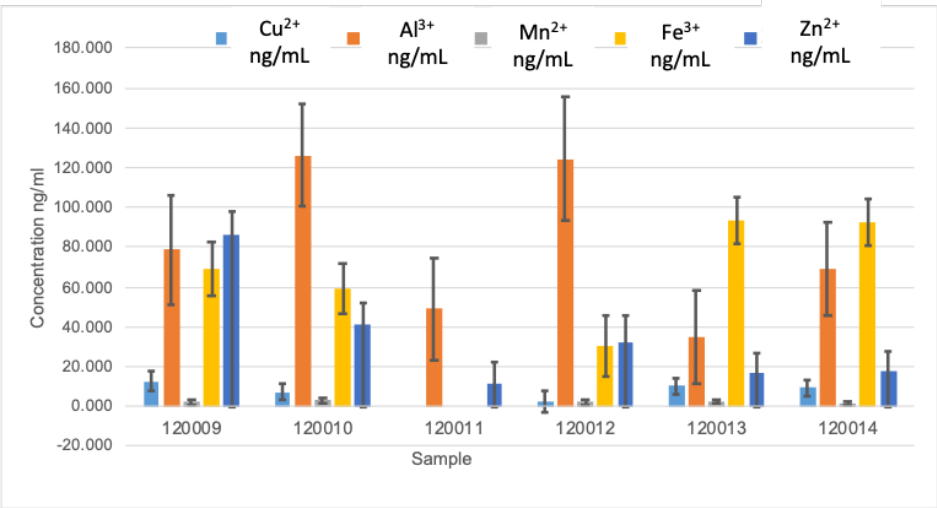


Figure 59 - ICP-MS concentrations for Cu^{2+}, Al^{3+}, Mn^{2+}, Fe^{2+} and Zn^{2+} in ng/mL in samples 120009 - 120014

The results indicate that using a full digestion method for the sample and cotton wool gave more consistent results. Nitric acid digestion and analysis via ICP-MS gave more consistent results. This was used to analyse nasal mucus for Cu^{2+}, Al^{3+}, Mn^{2+}, Fe^{2+} and Zn^{2+} moving forward.

Table 103 – *ICP-MS results for trace metal content in nasal mucus samples 120009 -120014 following nitric acid digestion, concentration in ng/mL and µmol/mL*

	Cu^{2+} (ng/ml)	SD	umol	SD	Al^{3+} (ng/ml)	SD	umol	SD	Mn^{2+} (ng/ml)	SD	umol	SD	Fe^{3+} (ng/ml)	SD	umol	SD	Zn^{2+} (ng/ml)	SD	umol	SD
120009	12.784	4.753	0.201	0.075	78.675	27.461	2.916	1.018	2.214	1.291	0.040	0.024	69.348	13.617	1.242	0.244	86.644	11.505	1.325	0.176
120010	7.415	4.414	0.117	0.069	126.256	25.499	4.680	0.945	3.036	1.199	0.055	0.022	59.581	12.644	1.067	0.226	41.662	10.684	0.637	0.163
120011	-0.066	4.414	-0.001	0.069	49.145	25.499	1.822	0.945	-0.790	1.199	-0.014	0.022	-18.577	12.644	-0.333	0.226	11.591	10.684	0.177	0.163
120012	2.442	5.433	0.038	0.085	124.403	31.384	4.611	1.163	2.105	1.476	0.038	0.027	30.467	15.562	0.546	0.279	32.372	13.149	0.495	0.201
120013	10.522	4.074	0.166	0.064	34.915	23.538	1.294	0.872	2.904	0.903	0.053	0.016	93.142	11.672	1.668	0.209	17.036	9.862	0.261	0.151
120014	9.508	4.074	0.150	0.064	69.302	23.538	2.569	0.872	1.732	0.903	0.032	0.016	92.652	11.672	1.659	0.209	17.532	9.862	0.268	0.151

5.2.3 **AAS, Nitric Acid Digestion, Bulk Metals**

AAS was selected to analyse the control samples and nasal mucus samples for the 4 bulk metals 10 cotton wool control samples seen in Table 104 and mucus samples 120009 to 120014 from section 5.2.2 were analysed.

Table 104 - Cotton wool control samples used in the analysis of bulk metals via AAS

Sample	Asda CW	Weight (g)	Nitric Acid (mL)
1	A	0.1306	2
2	B	0.1305	2
3	C	0.1308	2
4	D	0.1300	2
5	E	0.1307	2
6	F	0.1303	2
7	G	0.1304	2
8	H	0.1303	2
9	J	0.1307	2
10	K	0.1303	2
Mean		0.1305	
SD		0.0002	

Calibration standards were prepared in the range of blank to 100 mg/L for Na^+ and K^+, blank to 2 mg/L for Ca^{2+} and blank to 0.5 mg/L for Mg^{2+}.

The calibration curves can be seen in Appendix M.

The wavelengths and the machine range for analysis for the 4 bulk metals can be seen in Table 105 with the LOD and LOQ in Table 106.

Table 105 - Wavelengths and machine concentration ranges for AAS analysis

	Wavelength (nm)	Concentration Range (mg/L)	Gas
Na^+	330.2	0 - 400	Air/Acetylene
K^+	404.2	15 - 800	Air/Acetylene
Ca^{2+}	422.7	0.01 - 3	Nitrous Oxide/Acetylene
Mg^{2+}	285.2	0.003 - 1	Air/Acetylene

Table 106 - LOD and LOQ values for AAS analysis. Values are in mg/L

	LoD	LoQ
Na^+	0.172	0.839
K^+	3.52	12.708
Ca^{2+}	0.075	0.293
Mg^{2+}	0.092	0.289

The digested cotton wool supernatants were diluted 1:5 vol/vol with ultra-pure filtered water to bring them into the approximate range for AAS analysis. 10 control standards were analysed with an average mass of 0.1305 g of cotton wool in each sample. The results for the concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} in the diluted control samples can be seen in Table 107.

Table 107 – Concentrations for Na^+, K^+, Ca^{2+} and Mg^{2+}in the diluted digested cotton wool control samples

		Na^+			K^+			Ca^{2+}			Mg^{2+}		
Sample	CW Mass (g)	Conc mg/L	SD mg/L	%RSD	Conc mg/L	SD mg/L	%RSD	Conc mg/L	SD mg/L	%RSD	Conc mg/L	SD mg/L	%RSD
CW1	0.1306	7.26	0.09	1.2	1.37	1.42	28.9	0.70	0.02	2.9	0.22	0.04	16.6
CW2	0.1305	-0.80	0.18	50.8	0.69	0.65	15	0.49	0.01	2	0.30	0.02	7.5
CW3	0.1308	1.52	0.04	1.7	1.59	1.22	23.7	0.65	0.00	0.6	0.30	0.03	11.4
CW4	0.1300	-0.84	0.12	30.7	1.53	0.16	3.1	0.74	0.05	6.4	0.26	0.05	20.1
CW5	0.1307	1.33	0.09	4.9	2.20	1.82	31.5	0.60	0.01	1.4	0.27	0.03	10.2
CW6	0.1303	1.24	0.07	3.8	0.78	0.55	12.7	0.65	0.02	3.3	0.30	0.01	2.5
CW7	0.1304	1.34	0.12	6.1	1.37	1.25	25.1	0.55	0.02	3	0.30	0.06	20.4
CW8	0.1303	1.30	0.14	7.7	1.41	1.99	39.9	0.70	0.02	3.5	0.34	0.08	24.1
CW9	0.1307	1.28	0.21	12.1	1.29	1.38	28.2	0.63	0.02	3.1	0.28	0.02	7.9
CW10	0.1303	1.38	0.11	6	1.14	0.28	5.7	0.58	0.02	3	0.28	0.04	12.4
Mean	0.1305	1.34			1.34			0.63			0.28		
SD	0.0002	0.78			0.42			0.07			0.03		

The values in red were outliers. This was confirmed using the interquartile range calculation. The metal content values in the control samples will be used to give a representative value for the contribution made by the cotton wool in the mucus samples. The standardisation values can be seen in Table 108. The results, prior to adjustment for the allowance of metals attributed to cotton wool, can be seen in Table 109.

Table 108 - AAS standardisation values for Na^+, K^+, Ca^{2+} and Mg^{2+} to calculate the value attributed by the cotton wool splints in the nasal mucus samples

	Na^+		K^+		Ca^{2+}		Mg^{2+}	
CW Mass (g)	Conc mg/L	SD mg/L	Conc mg/L	SD mg/L	Conc mg/L	SD mg/L	Conc mg/L	SD mg/L
0.12	1.23	0.71	1.23	0.39	0.58	0.07	0.26	0.03
0.13	1.34	0.77	1.33	0.42	0.63	0.07	0.28	0.03
0.14	1.44	0.83	1.44	0.45	0.68	0.08	0.31	0.03
0.15	1.54	0.89	1.54	0.49	0.72	0.08	0.33	0.03
0.16	1.65	0.95	1.64	0.52	0.77	0.09	0.35	0.04

Table 109 - AAS results for Na^+, K^+, Ca^{2+} and Mg^{2+} prior to deduction of metals attributed to cotton wool or dilution factor

			Na^+		**K^+**		**Mg^{2+}**		**Ca^{2+}**	
Sample	**CW Mass (g)**	**Mucus Mass (g)**	**Conc mg/L**	**SD mg/L**	**Conc mg/L**	**SD mg/L**	**Conc mg/L**	**SD mg/L**	**Conc mg/L**	**SD mg/L**
120009	**0.1400**	**0.36**	19.0897	0.3399	5.1765	0.5391	0.4516	0.0255	2.0445	0.0412
120010	**0.1300**	**0.37**	19.6282	0.2127	5.2157	1.3262	0.5046	0.0398	9.3022	0.3247
120011	**0.1300**	**0.36**	16.3718	0.2893	5.0980	1.2372	0.4868	0.0491	3.2420	0.0207
120012	**0.1600**	**0.32**	17.2628	0.3924	8.0000	3.1933	0.5299	0.0413	2.4855	0.0301
120013	**0.1200**	**0.19**	10.7628	0.0909	4.8627	0.6968	0.3389	0.0056	1.2851	0.0077
120014	**0.1200**	**0.18**	10.7244	0.0675	3.2549	0.3240	0.3728	0.0117	1.2687	0.0535

Most of the results fell within the calibration range (section 5.3.6), however, K^+ was at the lower end of the calibration scale, Mg^{2+} at the top end of the calibration scale, with Ca^{2+} exceeding the top calibration value in 50% of the cases. The wavelength and calibration ranges were re-assessed for future sample analysis.

Concentrations for Na^+, K^+, Ca^{2+} and Mg^{2+} can be seen in mg/L In Table 110, and pictorially, in Figure 60, after the deduction for the cation contribution attributed to the cotton wool. The standardisation values can be seen in Table 111. The values have been factored up to the concentration in the mucus sample.

Table 110 – Bulk metal concentration in mg/L, allowing for metal content in cotton wool and refactored to allow for dilution

	Na^+ (mg/L)	K^+ (mg/L)	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)
120009	88.25	18.71	6.85	0.73
120010	91.46	19.42	43.38	1.10
120011	75.17	18.83	13.07	1.01
120012	78.09	31.80	8.57	0.90
120013	47.64	18.16	3.53	0.38
120014	47.45	10.12	3.45	0.55

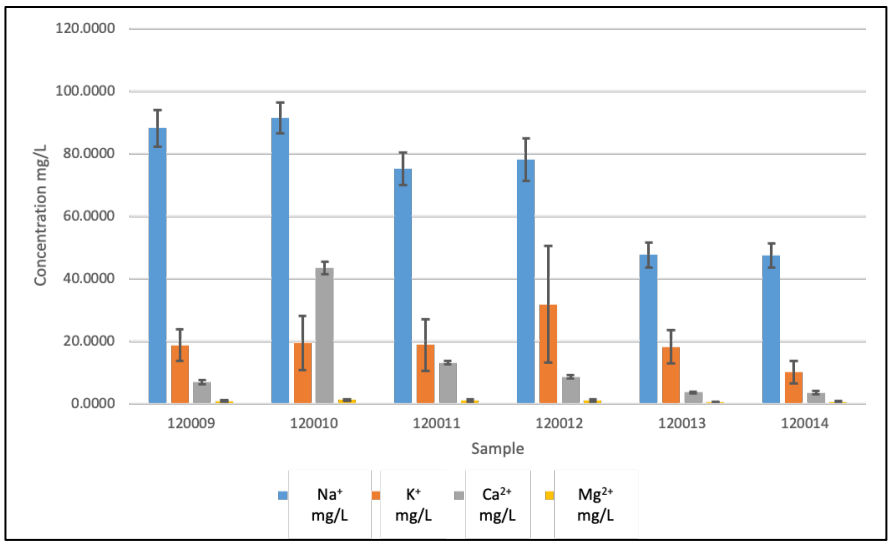


Figure 60 – Image displaying bulk metal concentration in mg/L, allowing for metal content in cotton wool and refactored to allow for dilution June 2019

The concentrations in mmol/L can also be seen in Table 111 and Figure 61.

Table 111 - Bulk metal concentration in mmol/L, allowing for metal content in cotton wool and refactored to allow for dilution

	Na^+ (mmol/L)	K^+ (mmol/L)	Ca^{2+} (mmol/L)	Mg^{2+} (mmol/L)
120009	3.84	0.48	0.17	0.03
120010	3.98	0.50	1.08	0.05
120011	3.27	0.48	0.33	0.04
120012	3.40	0.81	0.21	0.04
120013	2.07	0.46	0.09	0.02
120014	2.06	0.26	0.09	0.02

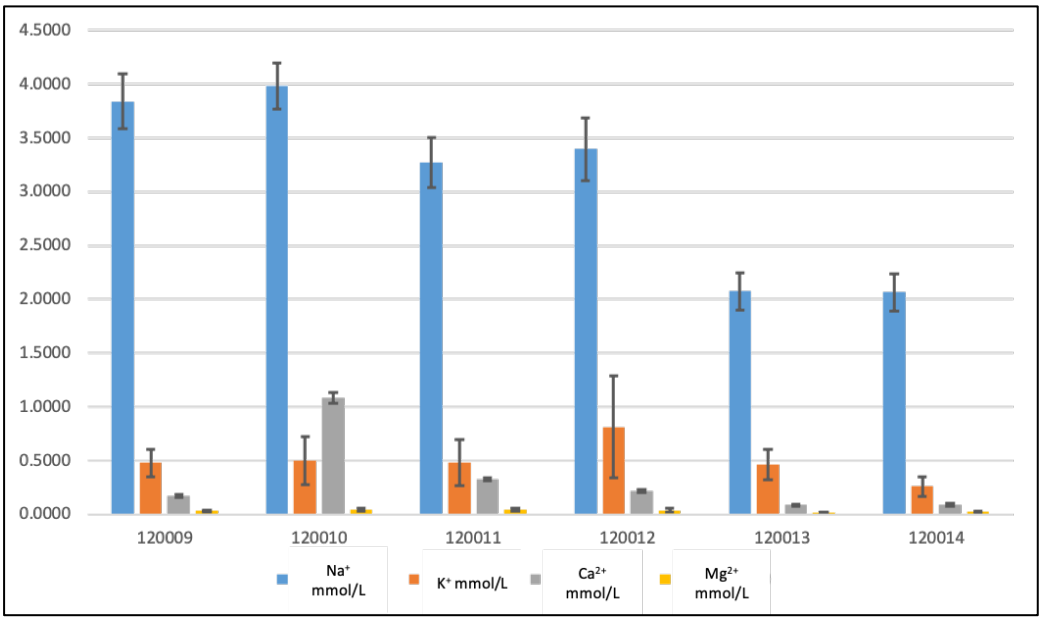


Figure 61 – Image displaying bulk metal concentration in mmol/L, allowing for metal content in cotton wool and refactored to allow for dilution

AAS was able to provide consistent results across the small sample set. A one-way ANOVA was used to analyse the results for left versus right nostril as seen in Table 112. The *p-value* indicates that the results are statistically similar.

Table 112 - One-way ANOVA showing AAS gives statistically similar results across the data set in method development

Anova: Single Factor					
SUMMARY					
Groups	*Count*	*Sum*	*Average*	*Variance*	*P-value*
Na R (mmol/L)	3	9.18	3.06	0.81	0.92
Na L (mmol/L)	3	9.44	3.15	0.96	
K R (mmol/L)	3	1.42	0.47	0.00	0.78
K L (mmol/L)	3	1.57	0.52	0.08	
Ca R (mmol/L)	3	0.59	0.20	0.01	0.45
Ca L (mmol/L)	3	1.38	0.46	0.29	
Mg R (mmol/L)	3	0.09	0.03	0.00	0.58
Mg L (mmol/L)	3	0.11	0.04	0.00	

5.3 Method Development Conclusion

The two methods of solubilisation were investigated using a range of analytical techniques. Cotton wool is highly absorbent which made it ideal to collect nasal mucus but was also made it difficult to separate nasal mucus from the cotton wool. Treating the cotton wool control samples with 5 or 10 mL of water gave inconsistent values for trace and bulk metal cation concentrations. It was not possible to calculate an accurate value for the cation element attributed to the cotton wool splint. This was necessary to analyse the cation content in the nasal mucus samples. Water solubilisation was discounted as a viable solution.

The second method of nitric acid digestion gave repeatable results on the cotton wool control samples. This enabled a standardised vale to be calculated for the concentration attributed to the cotton wool pellet. Nitric acid digestion was selected to be the method of choice to analyse nasal mucus samples for bulk and trace metal cations.

5.4 Experimental Method Development

5.4.1 Nasal Mucus Solubilisation - Water

A method of solubilisation was required to allow the cations/trace metals to be separated from the cotton wool and the mucus. One of the objectives was to quantify levels of Na^+ present in nasal mucus. Using a saline solution would solubilise the mucus, but it would introduce further Na^+ ions causing interference in an already complex matrix. Initially ultra-pure water was trialled as a solubilisation method. The method used can be seen in Figure 62 samples were analysed using ICP-MS at Aberystwyth University and IC at Bangor University.

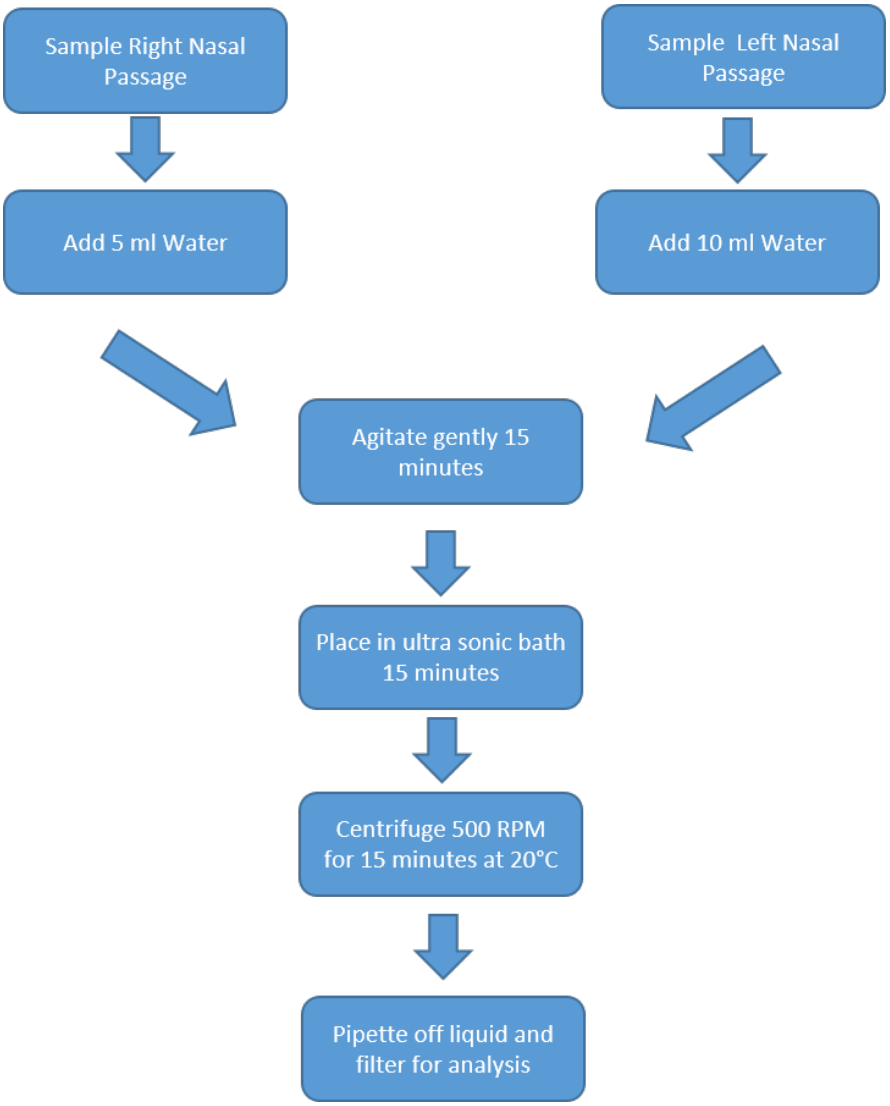


Figure 62 – Solubilisation of nasal mucus – water dilution process flow

5.4.2 **Mucus Solubilisation – Nitric Acid Digestion**

An alternative to water solubilisation of the mucus was a complete digestion of the cotton wool and the mucus in 68% nitric acid. The process flow can be seen in Figure 63.

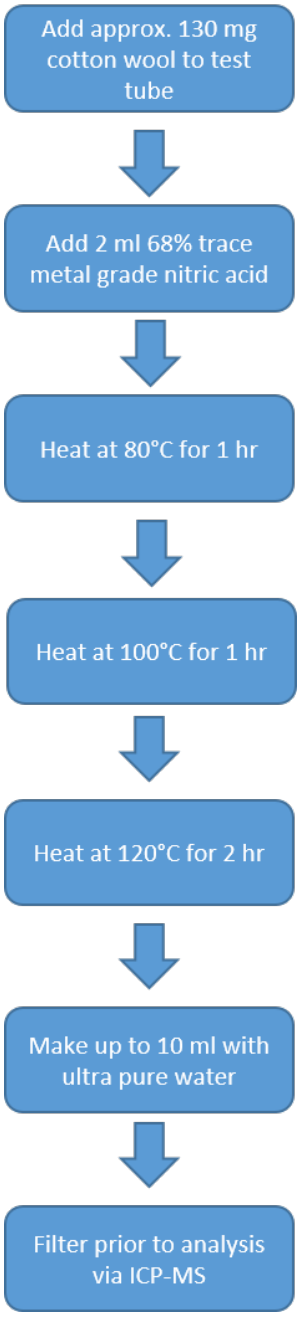


Figure 63 - Nitric acid digestion process

Both solubility methods were selected: a mild method using ultra-pure water and a full digestion method using 68% nitric acid. The samples were then analysed using inductively coupled plasma mass spectrometry (ICP-MS), ion chromatography (IC) and atomic absorption spectroscopy (AAS). The data obtained from each was used to determine which method of solubilisation and mode of analysis would be used moving forward. The results gave indicative values for trace and bulk metals in nasal mucus samples and cotton wool pellets.

Sample vials containing cotton wool with 5 or 10 mL ultra-pure water or 2 mL nitric acid were prepared and analysed alongside the nasal mucus samples to understand the bulk and trace metal contribution from the cotton wool.

Control samples of cotton wool (of approximately the same mass used in mucus collections), and mucus samples were treated using the method seen in Figure 62. Samples were analysed using ICP-MS, IC and AAS where applicable.

The second method was a complete digestion of the cotton wool containing the mucus sample using 68% nitric acid. Nasal mucus samples were suspended on cotton wool of approximately 130 mg in mass. Both the mucus and cotton wool control samples followed the process flow outlined in Figure 63.

5.4.3 ICP-MS Water Solubilisation Samples 120001 to 120008

All standards were prepared by the lab technician at Aberystwyth University. Standards were prepared for $Al^{3+,}$ Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+} were recorded. Al^{3+}, Fe^{3+}, and Mn^{2+} were recorded in helium mode.

10 control samples were prepared using approximately 130 mg of cotton wool (a similar mass to those used in the collection of nasal mucus). All samples were fully digested in 2 mL of 68% nitric acid over during a 4 h period where the temperature increase from 80 to 120 °C. The digestion solution was added to a 10 mL volumetric flask, made up to 10 mL with pure water, syringe filtered using a 0.2-micron filter, prior to analysis. The range of calibration standards used in the analysis can be seen in Table 113.

5.4.4 ICP-MS Samples Nitric Acid Digestion Samples 120009 - 1200014

The range of calibration standards used in the analysis can be seen in Table 114.

Table 113 - Calibration standards used in initial mucus ICP-MS analysis

			27 Al [tune 1]		27 Al [He mode]		55 Mn [He mode]		56 Fe [He mode]		63 Cu [tune 1]		66 Zn [tune 1]	
Type	Level	Sample Name	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD
CalBlk	1	Blk	0.000	N/A	0.000	N/A	0.000	N/A	0.000	N/A	0.000	N/A	0.000	N/A
CalStd	3	10 ng/mL	14.377	0.894	13.460	3.496	9.652	2.348	6.669	6.537	9.913	1.220	6.812	0.620
CalStd	4	20 ng/ml	24.648	0.775	24.285	1.719	20.390	1.155	18.079	1.373	22.688	0.783	19.943	2.434
CalStd	5	50 ng/mL	52.597	0.926	52.370	5.137	51.462	3.777	51.587	3.395	54.888	1.760	52.752	2.845
CalStd	6	100 ng/mL	100.448	2.205	95.365	1.476	94.936	1.975	97.295	3.649	102.198	1.546	101.640	4.192
CalStd	7	200 ng/mL	198.443	4.040	201.124	1.381	202.145	0.704	201.314	1.679	197.414	0.021	198.657	0.512

Table 114 - Calibration standards used in the 2nd mucus ICP-MS analysis

			27 Al [He mode]		55 Mn [He mode]		56 Fe [He mode]		63 Cu [tune 1]		66 Zn [tune 1]	
Type	Level	Sample Name	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD	Conc. [ng/ml]	Conc. RSD
CalBlk	1	Blank	0	N/A	0	N/A	0	N/A	0	N/A	0	N/A
CalStd	3	10 ng/mL	12.13906087	3.687862988	8.932488505	3.453235976	6.767097178	5.206864736	9.561304	0.68254599	6.151872488	5.977828042
CalStd	4	20 ng/ml	27.22645701	5.202482905	19.99736784	0.990884223	19.87460666	1.80292634	22.62057073	1.019403331	20.37200642	0.342682362
CalStd	5	50 ng/mL	50.62884195	0.424732073	48.76939966	1.76376982	49.57549884	2.857475425	52.05299085	1.644085646	51.04591812	1.448394897
CalStd	6	100 ng/mL	100.7567716	0.358387983	98.81749252	0.937436541	101.5132251	1.008889695	99.87099031	0.415118564	100.829324	0.504341279
CalStd	7	200 ng/mL	198.6348049	1.01291577	200.9525426	0.312247077	199.5236972	1.491518648	199.3111349	0.425414643	199.4790642	1.302392731

Nasal mucus samples 120009 - 120014, 3 pairs, suspended on cotton wool, were fully digested in 2 mL of 68% nitric acid over during a 4 h period with the temperature increasing from 80 to 120 °C. The digested solution was added to a 10 mL volumetric flask, made up to 10 mL with ultra-pure water and syringe filtered using a 0.2-micron filter, prior to analysis.

5.4.5 **IC Samples 120001 - 120008**

All Chemicals were purchased from Sigma Aldrich or Fisher Scientific. All chemicals were used as directed.

A small subset of samples were analysed to investigate the effectiveness of the method. A multi-ion standard was prepared from certified reference material TraceCERT®, 1000 mg/L Na^+ in water, 1000 mg/L K^+ in water, 1000 mg/L Ca^{2+} in nitric acid, and Mg^{2+} in nitric acid. Individual calibration standards were used to enable calibration at lower concentrations for K^+, Mg^{2+} and Ca^{2+}. It was unclear what concentrations would be seen in the mucus samples; therefore, standards were produced covering a wide range of concentrations.

The calibration standards can be seen in Table 115.

Table 115 - IC Calibration concentrations using individual standards March 2019

	Ret Time		Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7
Na^+	10.95	Lin	1	10	50	75	100	150	200
K^+	18.75	Lin	0.25	2.5	12.5	18.75	25	37.5	50
Mg^{2+}	20.57	Lin	0.05	0.5	2.5	3.75	5	7.5	10
Ca^{2+}	27.743	Lin	0.05	0.5	2.5	3.75	5	7.5	

5.4.6 **AAS Samples 120009 - 120014**

All chemicals were purchased from Sigma Aldrich and were used as directed.

Calibration standards were prepared from TraceCERT®, 1000 mg/L Na^+ in HNO_3 (1M), 1000 mg/L K^+ in HNO_3 (1M), 1000 mg/L Mg^{2+} in HNO_3 (1M), 1000 mg/L Ca^{2+} in HNO_3. Dilution was with ultra-pure water.

The nasal mucus samples were diluted 1:5, 2 mL of sample with 8 mL of ultra-pure filtered water to bring them into the approximate range for AAS analysis. 6 samples, 3

pairs, were analysed with the odd number representing the right nostril and the even representing the left nostril. A mall subset was used to gauge the suitability of AAS to analyse nasal mucus samples for bulk metal cations.

The calibration standards and absorption readings for Na^+ can be seen in Table 116.

Table 116 - Calibration data for Na^+, AAS analysis

Sample	Conc mg/L	%RSD	Mean	SD	Abs Readings		
ZERO	0.00	5.80	0.0023	0.0002	0.0022	0.0022	0.0025
1.00	5.00	2.20	0.0276	0.0006	0.0282	0.0271	0.0274
2.00	10.00	3.00	0.0542	0.0016	0.0526	0.0541	0.0558
3.00	25.00	1.60	0.1318	0.0022	0.1298	0.1316	0.1341
4.00	50.00	3.90	0.2598	0.0102	0.2565	0.2713	0.2517
5.00	75.00	0.80	0.3720	0.0031	0.3687	0.3749	0.3725
6.00	100.00	1.50	0.4827	0.0073	0.4886	0.4745	0.4850

The calibration standards and absorption readings for K^+ can be seen in Table 117.

Table 117 - Calibration data for K^+, AAS analysis

Sample	Conc mg/L	%RSD	Mean	SD	Abs Readings		
Zero	0	28.4	0.0074	0.0021	0.0096	0.007	0.0055
1.00	15	12.1	0.0313	0.0038	0.028	0.0354	0.0304
2.00	25	1.9	0.0455	0.0009	0.0465	0.0448	0.0452
3.00	50	1.6	0.0871	0.0014	0.0855	0.0879	0.0879
4.00	75	4.9	0.1333	0.0065	0.1294	0.1408	0.1298
5.00	100	3.1	0.1704	0.0053	0.1765	0.1684	0.1664

The calibration standards and absorption readings for Ca^{2+} can be seen in Table 118.

Table 118 - Calibration data for Ca^{2+}, AAS analysis

Sample	Conc mg/L	%RSD	Mean	SD	Abs Readings		
ZERO	0	43.6	0.0018	0.0008	0.0018	0.001	0.0025
1.00	0.5	6.1	0.0240	0.0015	0.0242	0.0253	0.0224
2.00	0.75	0.4	0.0328	0.0002	0.0328	0.0327	0.033
3.00	1	4	0.0433	0.0017	0.0452	0.0418	0.043
4.00	1.25	3.5	0.0542	0.0019	0.0528	0.0563	0.0534
5.00	1.5	1.5	0.0670	0.0010	0.0671	0.0679	0.0659
6.00	2	10.2	0.0927	0.0095	0.0877	0.0867	0.1036

The calibration standards and absorption readings for Mg^{2+} can be seen in Table 119.

Table 119 - Calibration data for Mg^{2+}, AAS analysis

Sample	Conc mg/L	%RSD	Mean	SD	Abs Readings		
ZERO	0	2.2	0.0000	0.0006	0.0246	0.0236	0.0245
1.00	0.15	17.2	0.0090	0.0016	0.0106	0.0088	0.0075
2.00	0.2	2.6	0.0113	0.0003	0.0111	0.0117	0.0112
3.00	0.25	10.7	0.0132	0.0014	0.0142	0.0139	0.0116
4.00	0.3	11.9	0.0172	0.0020	0.0166	0.0194	0.0155
5.00	0.4	6.6	0.0210	0.0014	0.021	0.0197	0.0224
6.00	0.5	11.4	0.0277	0.0032	0.0283	0.0306	0.0243

6 Nasal Mucus Analysis in Healthy Participants

One of the aims of this study was to gain a greater understanding of the composition of nasal mucus and how it varies in the healthy adult population. Previous studies in section 1.8 had looked at Na^+, K^+, Ca^{2+}, and Mg^{2+} cation concentrations, but there have been limited investigations into trace metal cations. This study is looking at the bulk metal cations and trace metal elements Al^{3+}, Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+} to see if there is any correlation between the levels of these ions in nasal mucus. Healthy volunteers, 20 males and 20 females, were recruited from the student community at Bangor University.

6.1 Results and Discussion Trace Metal Analysis of Cotton Wool and Nasal Mucus ICP-MS

The LOD and LOQ for the 5 trace metals of interest can be seen in Table 120.

Table 120 - LOD and LOQ values for Al^{3+}, Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+} via ICP-MS in ng/mL

	Al^{3+}	Cu^{2+}	Fe^{3+}	Mn^{2+}	Zn^{2+}
LoD ng/ml	1.132	0.073	0.111	0.007	0.286
LoQ ng/ml	2.668	1.617	8.114	0.217	2.915

20 control samples, cotton wool digested in nitric acid, had been prepared to give an approximation of the cation concentrations in the cotton wool splints. The average mass of the cotton wool control samples used was 0.13 g, a similar value to that used in the collection of nasal mucus. The average concentration values of the 5 ions of interest in the cotton wool can be seen in Table 121.

As the mass of the cotton wool splints that were used in the sample analysis varied, these values were used to give a representative value for the mass of the cotton wool used to collect each individual sample. The values in Table 122 were deducted from the digested sample and cotton wool sample concentrations to give an ion concentration in the nasal mucus sample.

Table 121 - Average ion concentrations for Al^{3+}, Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+} in ng/mL, in 0.13 g of cotton wool

	Avg mass of cotton wool (g)	SD (g)	Concentration (ng/ml)	SD (ng/ml)
Al^{3+}	0.1308	0.0008	311.2	41.7
Cu^{2+}	0.1308	0.0008	6.9	1.5
Fe^{3+}	0.1308	0.0008	236.5	18.2
Mn^{2+}	0.1308	0.0008	24.5	1.3
Zn^{2+}	0.1308	0.0008	37.4	9.3

Table 122 - The standardised values for the trace metal ions in cotton wool with standard deviation

Mass Cotton Wool (g)	Al^{3+} (ng/mL)	SD	Cu^{+} (ng/mL)	SD	Fe^{3+} (ng/mL)	SD	Mn^{2+} (ng/mL)	SD	Zn^{2+} (ng/mL)	SD
0.10	238.0	31.9	5.0	0.8	177.5	9.4	18.7	1.0	28.6	7.1
0.11	261.8	35.0	5.5	0.9	195.3	10.4	20.6	1.1	31.4	7.8
0.12	285.5	38.2	6.0	1.0	213.0	11.3	22.5	1.2	34.3	8.5
0.13	309.3	41.4	6.5	1.1	230.8	12.2	24.3	1.3	37.2	9.2
0.14	333.1	44.6	7.0	1.1	248.5	13.2	26.2	1.4	40.0	10.0
0.15	356.9	47.8	7.5	1.2	266.3	14.1	28.1	1.5	42.9	10.7
0.22	523.5	70.1	11.0	1.8	390.5	20.7	41.2	2.3	62.9	15.6

These values were deducted from the overall cation values for the volunteer samples to give the ionic concentration in the mucus samples.

The ion concentration results for Al^{3+}, Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+} in the 80 nasal mucus samples can be seen in the tables in Appendix P and will be discussed in section 6.3. The tables show the mass of cotton wool used in each sample with the cation content attributed to the mass of cotton wool. The concentration of the cation in the digested cotton wool and mucus sample along with the concentration of the cation in the original mucus sample. The values for the standardised concentration of each cation per g of mucus in ng/mL and in μmol/L in each sample can also be seen.

6.2 Bulk Metal Analysis of Cotton Wool and Nasal Mucus AAS

Calibration curves for Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+} can be seen in Appendix O.

The LOD and LOQ for the bulk metals can be seen in Table 123.

Table 123 - LOD and LOQ for Na^+, K^+, Ca^{2+}, and Mg^{2+} in mg/L

	Na^+	K^+	Mg^{2+}	Ca^{2+}
LOD	-0.03	0.108	0.001	0.055
LOQ	0.001	0.161	0.004	0.078

The average mass of the cotton wool control samples was 0.13 g. The average concentration values for the 4 ions can be seen in Table 124.

Table 124 - The average ion concentrations for Na^+, K^+, Ca^{2+}, and Mg^{2+} in 0.13 g of cotton wool

Ion	Avg Mass (g)	SD (g)	Concentration (g)	SD (g)
Na^+	0.1308	0.0008	0.03	0.006
K^+	0.1308	0.0008	0.27	0.007
Mg^{2+}	0.1308	0.0008	0.14	0.002
Ca^{2+}	0.1308	0.0008	0.39	0.010

As the mass of the cotton wool splints that were used in the sample analysis varied, these values were used to give a representative value for the mass of the cotton wool used to collect each individual sample. These values can be seen in Table 125.

Table 125 - The standardised values for the bulk ions in cotton wool with standard deviation

Standardised mass of cotton wool (g)	Na^+ (mg/L)	SD	K^+ (mg/L)	SD	Mg^{2+} (mg/L)	SD	Ca^{2+} (mg/L)	SD
0.10	0.03	0.005	0.21	0.006	0.11	0.002	0.30	0.008
0.11	0.03	0.005	0.23	0.006	0.12	0.002	0.32	0.008
0.12	0.03	0.005	0.25	0.007	0.13	0.002	0.35	0.009
0.13	0.03	0.006	0.27	0.007	0.14	0.002	0.38	0.010
0.14	0.04	0.006	0.29	0.008	0.15	0.003	0.41	0.105
0.15	0.04	0.007	0.31	0.008	0.16	0.003	0.44	0.011
0.22	0.06	0.010	0.46	0.012	0.24	0.004	0.65	0.016

The cation value for the mass of cotton wool was deducted from the overall cation values for the volunteer samples to give the ionic concentration in the mucus samples.

The results for the bulk metals in the nasal mucus samples were calculated. The concentration values for Na^+, K^+, Ca^{2+}, and Mg^{2+} can be seen in Appendix Q and will be discussed in detail in section 6.3. Each table shows the mass of cotton wool used

in each sample with the cation content attributed to the mass of cotton wool. The concentration of the cation in the digested cotton wool and mucus sample along with the concentration of the cation in the original mucus sample. The concentration is standardised to give the concentration of the cation per g of mucus in mg/L and also in mmol/L.

6.3 Statistical Analysis – Analysis of Cation Concentrations in Nasal Mucus.

In section 1.8, previous literature was interrogated. Only basic analysis was performed in these works. The similarities between male and female participants or between left and right nostril mucus collections was not investigated. This study has investigated these similarities and has also looked into the correlation between the 9 cations in nasal mucus. IBM SPSS[83] was chosen to process the data. The results for all 9 bulk and trace metals in the mucus samples were uploaded into SPSS for statistical analysis. The results were analysed to look for similarities and differences between the results for male and female participants, and for left and right. Descriptive analysis, correlation and one-way ANOVA reports were used for this purpose. Kurtosis values were used to investigate outliers in the dataset. A normal distribution would give a kurtosis of 3. This would give a bell curve that is symmetrical about the mean with no outliers. A kurtosis value greater than 3 gives a leptokurtic distribution. This distribution has broader tails which give rise to a larger number of extreme outliers. A distribution of less than 3 gives a platykurtic distribution. This gives rise to thinner tails and less possibility of extreme outliers.

A descriptive analysis report gave the details in Table 126 in mg/L and in Table 127 in mmol/L. The tables show the mean, median, range, standard deviation, standard error with the minimum and maximum values for the 9 bulk or trace metals. The kurtosis for Mg^{2+}, Ca^{2+}, Al^{3+}, Fe^{3+}, Mn^{2+} and Zn^{2+} is above 3. This makes the distribution leptokurtic. There is a likelihood of greater and more extreme outliers. The kurtosis for Na^{+}, K^{+} and Cu^{2+} is below 3. This makes the distribution platykurtic. You would expect to see fewer and less extreme outliers. Outlier analysis was performed on the data. The extreme values for each cation, the top 5 and bottom 5, can be seen in Table 128.

The case numbers relate to the sample ID's where case number 1 is sample ID 120015, case number 2 is sample 120016 etc.

In total, 19 outliers were identified. The outliers can be visualised on the box plots below. There were 2 outliers identified for Na^+, sample 3 and 56, relating to sample ID's 120017 and 120070. This can be seen in Figure 64.

Table 126 - Descriptive statistics for 80 mucus samples for all 9 trace and bulk metals in mg/L

Descriptive Statistics	N	Range	Minimum	Maximum	Mean		Std. Deviation	Variance	Kurtosis	
					Statistic	Std Error			Statistic	Std Error
Na^{+} conc mg/L per g mucus	80	2639.17	1322.80	3961.97	2990.38	48.26	431.63	186305.60	2.13	0.53
K^{+} conc mg/L per g mucus	80	1001.52	268.16	1269.68	614.72	18.07	161.60	26114.46	2.35	0.53
Mg^{2+} conc mg/L per g mucus	80	109.29	-6.73	102.56	22.11	1.54	13.77	189.57	14.00	0.53
Ca^{2+} conc mg/L per g mucus	80	2794.55	-111.83	2682.73	299.49	45.61	407.96	166433.38	21.88	0.53
Al^{3+} conc mg/L per g mucus	80	472.81	-2.06	470.75	15.74	5.93	53.08	2817.62	70.64	0.53
Cu^{2+} conc mg/L per g mucus	80	1.20	0.11	1.31	0.48	0.03	0.27	0.07	0.85	0.53
Fe^{3+} conc mg/L per g mucus	80	22.65	0.36	23.01	6.48	0.51	4.52	20.44	3.32	0.53
Mn^{2+} conc mg/L per g mucus	80	1.18	-0.47	0.70	0.09	0.02	0.15	0.02	5.32	0.53
Zn^{2+} conc mg/L per g mucus	80	41.13	0.31	41.44	2.45	0.56	4.99	24.93	49.84	0.53

Table 127 - Descriptive statistics for 80 mucus sample s for all 9 trace and bulk metals in mmol/L

Descriptive Statistics	N	Range	Minimum	Maximum	Mean		Std. Deviation	Variance	Kurtosis	
					Statistic	Std Error			Statistic	Std Error
Na^{+} conc mmol/L per g mucus	80	114.801	57.540	172.342	130.079	2.10	18.78	352.52	2.13	0.53
K^{+} conc mmol/L per g mucus	80	25.616	6.859	32.474	15.722	0.46	4.13	17.08	2.35	0.53
Mg^{2+} conc mmol/L per g mucus	80	4.497	-0.277	4.220	0.910	0.06	0.57	0.32	14.00	0.53
Ca^{2+} conc mmol/L per g mucus	80	69.728	-2.790	66.938	7.473	1.14	10.18	103.62	21.88	0.53
Al^{3+} conc mmol/L per g mucus	80	17.524	-0.076	17.448	0.583	0.22	1.97	3.87	70.64	0.53
Cu^{2+} conc mmol/L per g mucus	80	0.019	0.002	0.020	0.007	0.00	0.00	0.00	0.85	0.53
Fe^{3+} conc mmol/L per g mucus	80	0.406	0.006	0.412	0.116	0.01	0.08	0.01	3.32	0.53
Mn^{2+} conc mmol/L per g mucus	80	0.021	-0.009	0.013	0.002	0.00	0.00	0.00	5.32	0.53
Zn^{2+} conc mmol/L per g mucus	80	0.629	0.005	0.634	0.038	0.01	0.08	0.01	49.84	0.53

Table 128 - Extreme values identified during outlier analysis. Case numbers were assigned in SPSS with case number 1 as sample ID 120014 to case number 80 as sample ID 120094

Top and Bottom Extreme Concentration Values		Na^+ Case Number	Na^+ conc mg/L per g mucus	K^+ Case Number	K^+ conc mg/L per g mucus	Mg^{2+} Case Number	Mg^{2+} conc mg/L per g mucus	Ca^{2+} Case Number	Ca^{2+} conc mg/L per g mucus	Al^{3+} Case Number	Al^{3+} conc mg/L per g mucus	Cu^{2+} Case Number	Cu^{2+} conc mg/L per g mucus	Fe^{3+} Case Number	Fe^{3+} conc mg/L per g mucus	Mn^{2+} Case Number	Mn^{2+} conc mg/L per g mucus	Zn^{2+} Case Number	Zn^{2+} conc mg/L per g mucus
Highest	1	53	3961.97	53	1269.68	53	102.56	21	2682.73	56	470.75	38	1.31	63	23.01	32	0.70	6	41.44
	2	28	3871.53	65	964.99	21	47.10	27	2313.81	32	57.30	39	1.27	32	21.02	59	0.48	20	20.12
	3	38	3791.77	6	914.78	48	43.28	32	1409.74	59	55.22	53	1.14	59	19.86	21	0.44	34	5.30
	4	46	3719.71	63	890.50	32	41.88	28	1040.44	60	51.50	57	1.03	62	19.52	30	0.36	38	4.78
	5	34	3594.06	66	883.82	46	40.05	19	828.30	61	46.71	58	0.95	57	13.88	28	0.36	31	4.29
Lowest	1	56	1322.80	27	268.16	56	-6.73	1	-111.82	50	-2.06	67	0.11	1	0.36	6	-0.47	75	0.31
	2	3	1966.59	56	363.51	6	-5.31	56	15.14	1	-0.45	66	0.14	78	0.92	1	-0.18	65	0.35
	3	11	2160.64	11	386.52	78	-3.04	55	87.37	77	-0.20	69	0.15	77	1.14	11	-0.17	7	0.41
	4	1	2188.83	29	393.41	1	5.07	60	96.25	45	0.03	3	0.15	6	1.28	3	-0.08	77	0.49
	5	27	2336.49	79	417.08	3	6.88	78	101.38	71	0.21	61	0.17	75	1.32	78	-0.08	69	0.51

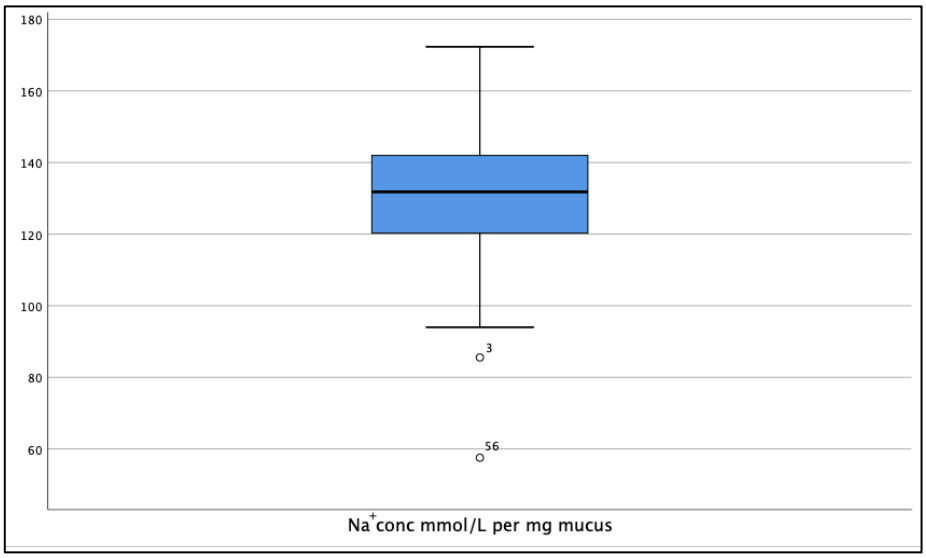


Figure 64 - Outlier box plot generated in SPSS for Na^+ identifying case number 3 and 56

There was only 1 outlier for K^+, and this can be seen in Figure 65. This is case number 53 and relates to sample ID 120067.

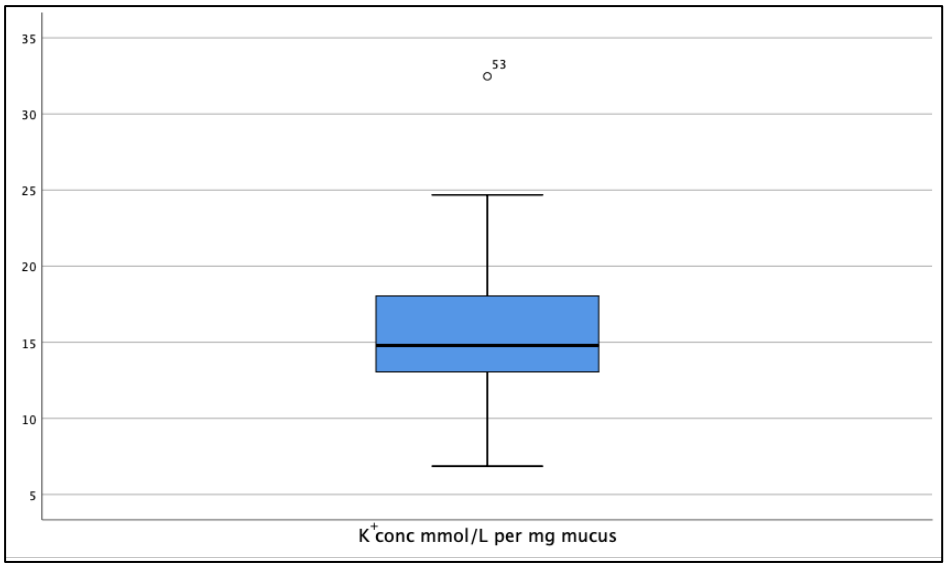


Figure 65 - Outlier box plot generated in SPSS for K^+ identifying case number 53

There were 3 outliers for Mg^{2+}, case numbers 6 and 56, sample ID's 120020 and 120070 respectively, both below the mean, and case number 53 sample ID 120067, an extreme outlier above the mean. This can be seen in the boxplot in Figure 66.

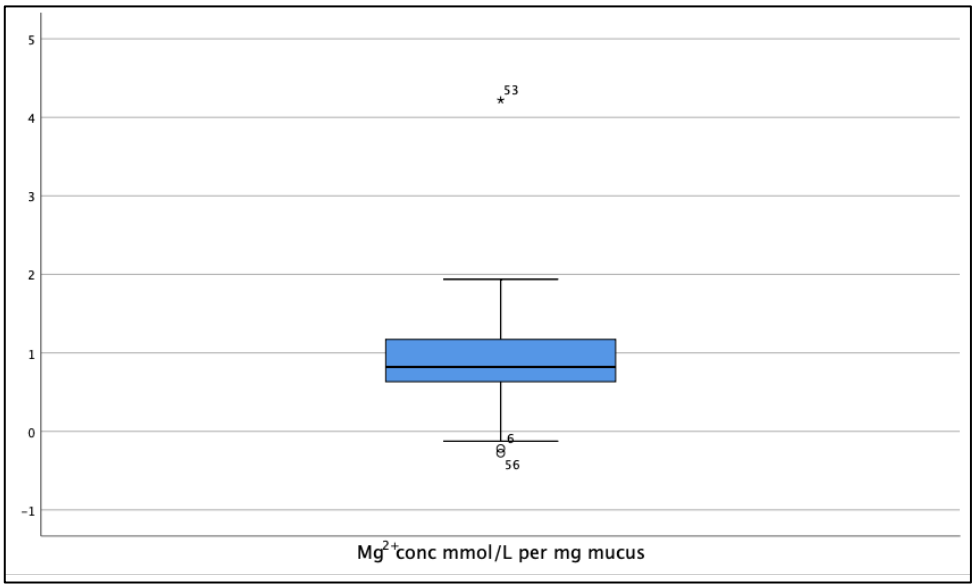


Figure 66 - Outlier box plot generated in SPSS for Mg^{2+} identifying case numbers 6, 53 and 56

There were multiple outliers for Ca^{2+}, all but one is above the mean. Case number 1, sample ID 120015 falls below the mean. Details of the outliers can be seen in Table 129 and in the box plot in Figure 67.

Table 129 - Ca^{2+} outliers, case numbers and sample ID's

Case No	Sample ID
19	120033
21	120035
27	120041
28	120042
32	120046
53	120067
1	120015

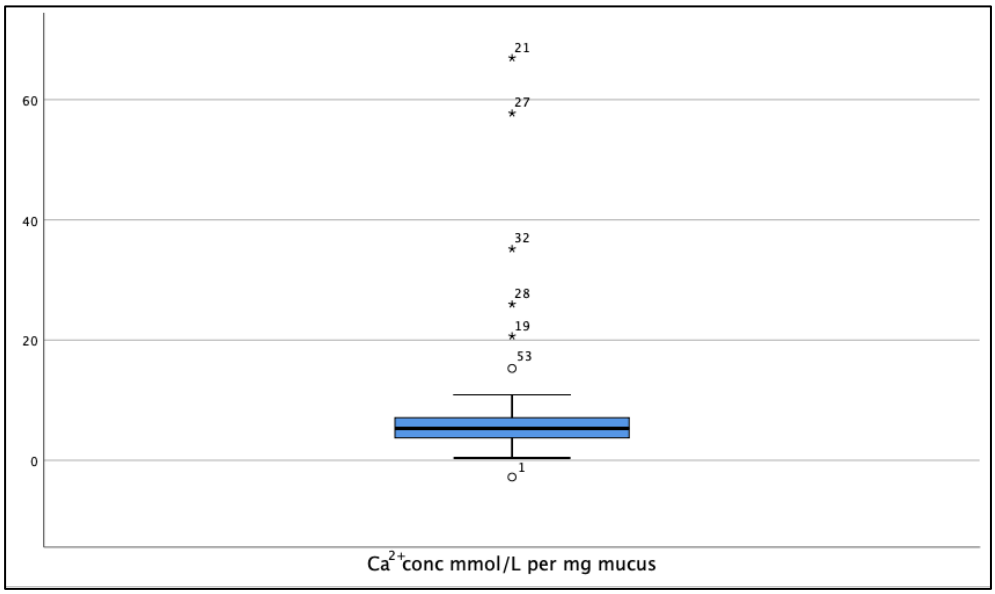


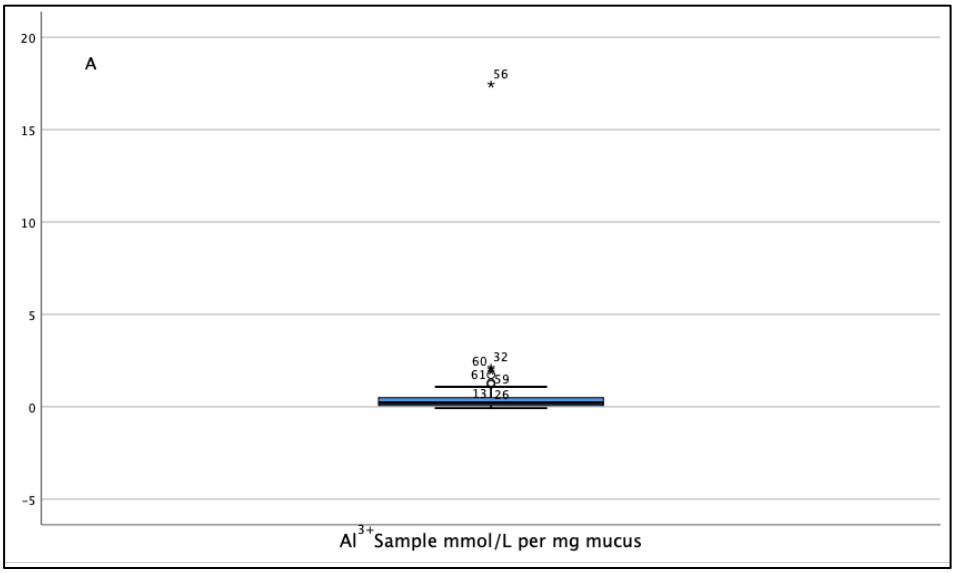
Figure 67 - Outlier box plot generated in SPSS for Ca^{2+} identifying case numbers 1, 19, 21, 27, 28, 32 and 53

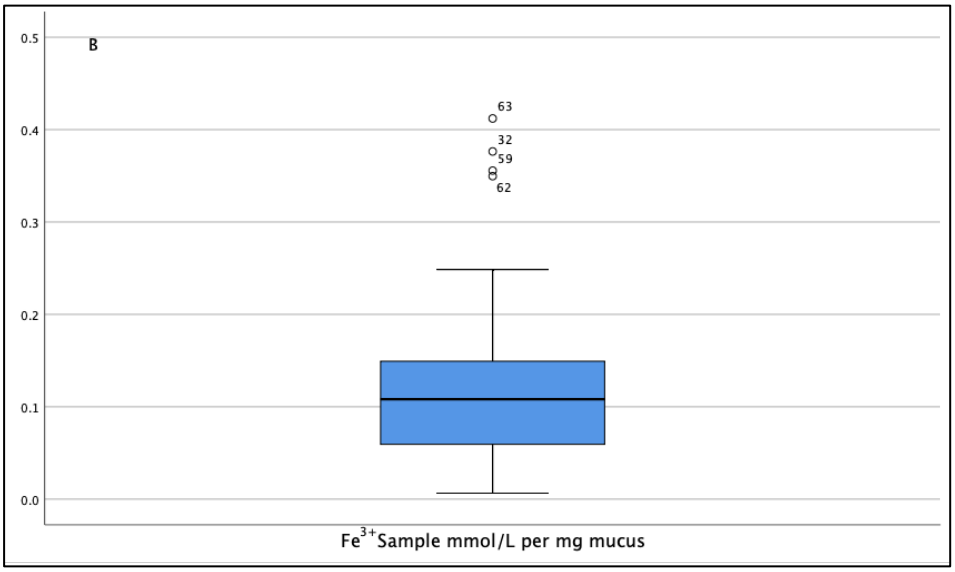
There were a greater number of outliers within the trace metals. The values for the 5 trace metals can be seen in Table 130. The outliers above the mean can be seen in green and those below the mean in yellow.

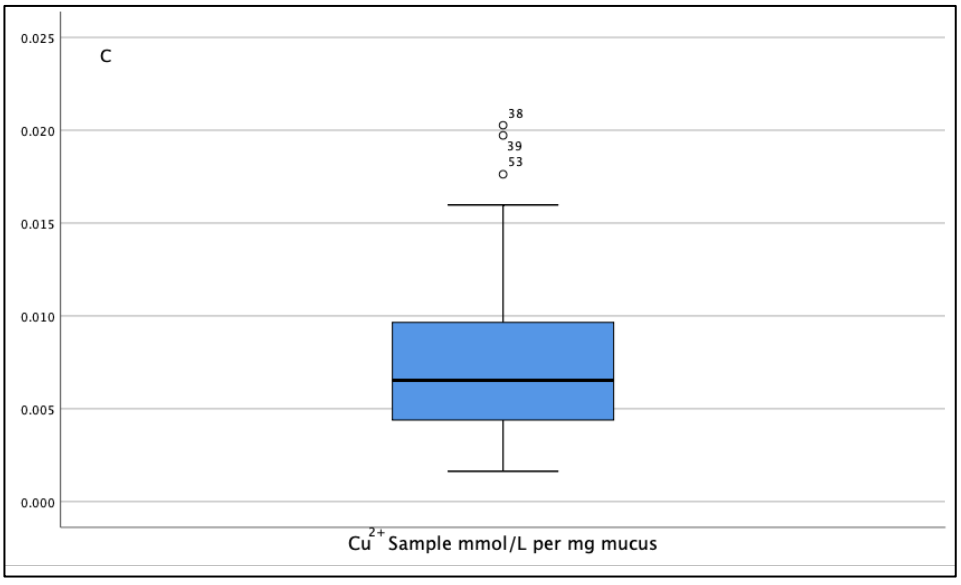
Table 130 - Outliers for the 5 trace metals displaying case numbers and sample ID's, the values in green fall below the mean and in yellow above the mean

Case Number	Sample ID	Al^{3+}	Cu^{2+}	Fe^{3+}	Mn^{2+}	Zn^{2+}
1	120015				Yes	
3	120017					
6	120020				Yes	Yes
19	120033					
20	120034					Yes
21	120035				Yes	
27	120041					
28	120042				Yes	
30	120044				Yes	
32	120046	Yes		Yes	Yes	
38	120052		Yes			
39	120053		Yes			
53	120067		Yes			
56	120070	Yes				
59	120073	Yes		Yes	Yes	
60	120074	Yes				
61	120075	Yes				
63	120077			Yes		
78	120092				Yes	

The boxplots for the 5 trace metals can be seen in Figure 68.







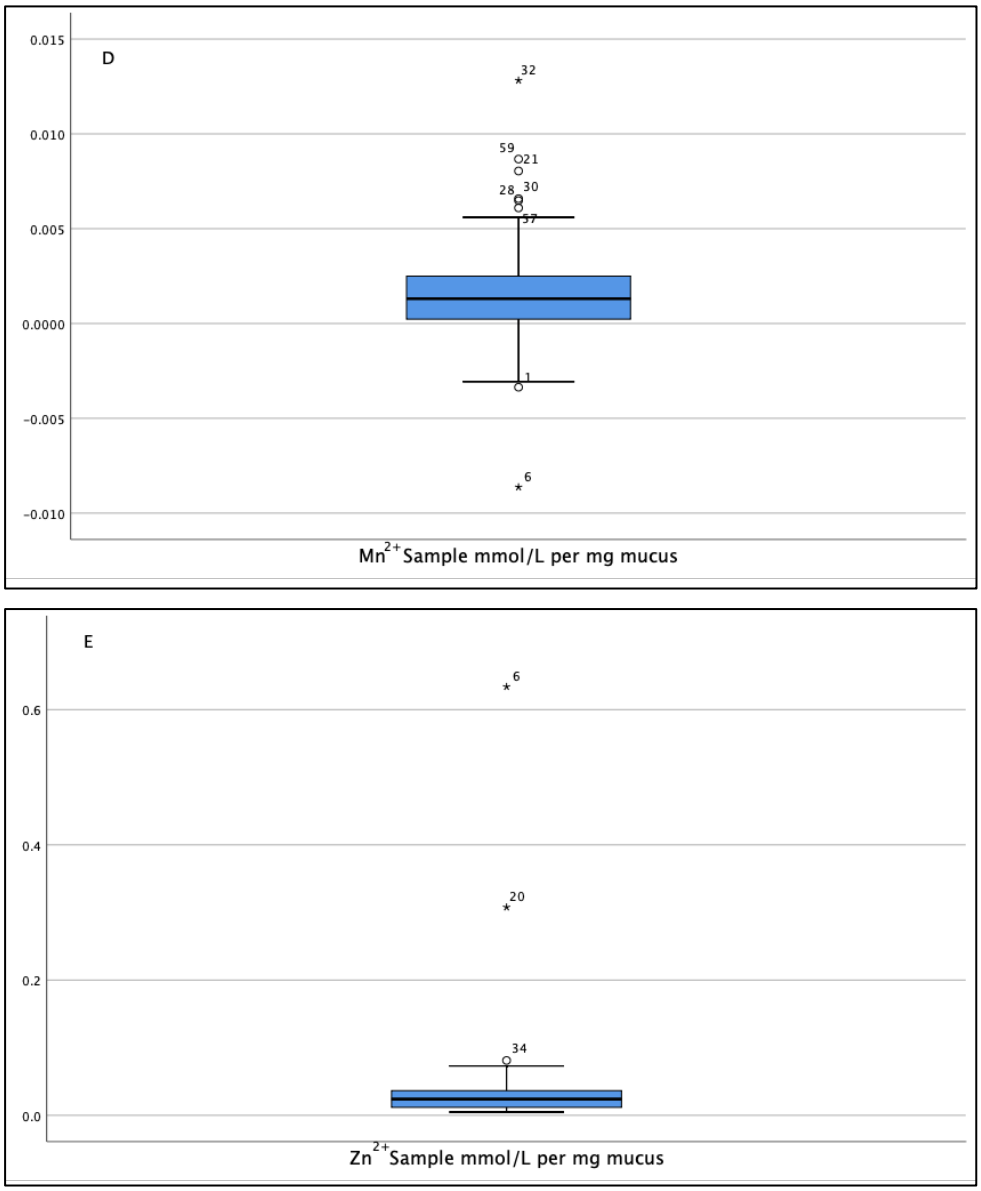


Figure 68 - Boxplots depicting outliers for the 5 trace metals depicting Al^{3+} (A), Fe^{3+} (B), Cu^{2+} (C), Mn^{2+} (D) and Zn^{2+} (E)

Out of the 19 outlier samples, 3 were paired samples. Pair sample ID's 120041 and 120042 were both contained a high Ca^{2+} concentration. This may be due to analysis error or may be due to an undiagnosed medical problem. Sample ID's 120033 and 120034 both had an individual outlier, but all other cations were within normal ranges. Sample ID's 120073 and 120074 both had high values for Al^{3+}. Al^{3+} is not a biological trace metal. Al^{3+} should not be present in the human system.[84] It is unclear if the Al^{3+} present in nasal mucus is environmental. It may be breathed in the air, as a result of vaping or from aerosol spray deodorants. The other 13 single outliers were high and low across multiple elements. The corresponding paired samples were all within

normal acceptable ranges. These samples will be discussed in more detail later in this section. The outlier samples may not have digested fully, resulting in lower than anticipated concentrations. Table 131 shows all the outliers. The upper outliers can be seen in red, lower outliers in blue, values within the top 5 concentrations in green and values within the bottom 5 concentrations in yellow. Most outliers have values within these areas.

Table 131 - All 19 samples containing outliers. The upper outliers can be seen in red, lower outliers in blue, values within the top 5 concentrations in green and values within the bottom 5 concentrations in yellow. Most outliers have values within these areas.

	Sample ID	Na^{+}	K^{+}	Mg^{2+}	Ca^{2+}	Al^{3+}	Cu^{2+}	Fe^{3+}	Mn^{2+}	Zn^{2+}
1	120015								Yes	
3	120017	Yes			Yes					
6	120020			Yes					Yes	Yes
19	120033				Yes					
20	120034									Yes
21	120035				Yes				Yes	
27	120041				Yes					
28	120042				Yes				Yes	
30	120044								Yes	
32	120046				Yes	Yes		Yes	Yes	
38	120052						Yes			
39	120053						Yes			
53	120067		Yes	Yes	Yes		Yes			
56	120070	Yes		Yes		Yes				
59	120073					Yes		Yes	Yes	
60	120074					Yes				
61	120075					Yes				
63	120077							Yes		
78	120092								Yes	

	extreme upper outlier
	extreme Lower outlier
	bottom 5 extreme numbers
	top 5 extreme numbers

The 19 samples containing outliers were removed from the dataset and statistical analysis performed on the remaining data. The descriptive statistics can be seen in Table 132 in mg/L and in Table 133 in mmol/L.

The values for the bulk metals fall broadly in line with values seen in previous research, however, there has been limited research carried out into trace metals in nasal mucus. The values were found for Zn^{2+} and Cu^{2+} in one study, but no information was found regarding Mn^{2+}, Fe^{3+} or Al^{3+}. These can be seen in section 1.9 and Table 7.

Table 132 - Descriptive statistics for the subset of 61 samples in mg/L

Descriptive Statistics	N	Range	Minimum	Maximum	Mean		Std. Deviation	Variance	Kurtosis	
					Statistic	Std Error			Statistic	Std Error
Na^{+} conc mg/L per g mucus	61	1559.08	2160.64	3719.71	3003.15	43.29	338.07	114294.43	-0.40	0.60
K^{+} conc mg/L per g mucus	61	578.47	386.52	964.99	619.13	17.76	138.68	19231.60	-0.54	0.60
Mg^{2+} conc mg/L per g mucus	61	33.16	10.12	43.28	21.70	1.01	7.89	62.21	0.22	0.60
Ca^{2+} conc mg/L per g mucus	61	349.67	87.37	437.03	208.30	9.64	75.31	5672.07	0.59	0.60
Al^{3+} conc mg/L per g mucus	61	37.13	-2.06	35.07	7.28	1.10	8.58	73.70	2.63	0.60
Cu^{2+} conc mg/L per g mucus	61	0.93	0.11	1.03	0.45	0.03	0.23	0.05	-0.12	0.60
Fe^{3+} conc mg/L per g mucus	61	18.38	1.14	19.52	5.98	0.45	3.48	12.13	2.49	0.60
Mn^{2+} conc mg/L per g mucus	61	0.50	-0.17	0.33	0.08	0.01	0.09	0.01	0.84	0.60
Zn^{2+} conc mg/L per g mucus	61	4.99	0.31	5.30	1.68	0.14	1.10	1.20	1.38	0.60

Table 133 - Descriptive statistics for the subset of 61 samples in mmol/L

Descriptive Statistics	N	Range	Minimum	Maximum	Mean		Std. Deviation	Variance	Kurtosis	
					Statistic	Std Error			Statistic	Std Error
Na^{+} conc mmol/L per g mucus	61	67.82	93.99	161.80	130.63	1.88	14.71	216.26	-0.40	0.60
K^{+} conc mmol/L per g mucus	61	14.80	9.89	24.68	15.84	0.45	3.55	12.58	-0.54	0.60
Mg^{2+} conc mmol/L per g mucus	61	1.36	0.42	1.78	0.89	0.04	0.32	0.11	0.22	0.60
Ca^{2+} conc mmol/L per g mucus	61	8.72	2.18	10.90	5.20	0.24	1.88	3.53	0.59	0.60
Al^{3+} conc mmol/L per g mucus	61	1.38	-0.08	1.30	0.27	0.04	0.32	0.10	2.63	0.60
Cu^{2+} conc mmol/L per g mucus	61	0.01	0.00	0.02	0.01	0.00	0.00	0.00	-0.12	0.60
Fe^{3+} conc mmol/L per g mucus	61	0.33	0.02	0.35	0.11	0.01	0.06	0.00	2.49	0.60
Mn^{2+} conc mmol/L per g mucus	61	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.84	0.60
Zn^{2+} conc mmol/L per g mucus	61	0.08	0.00	0.08	0.03	0.00	0.02	0.00	1.38	0.60

6.3.1 **Analysis of Nasal Mucus: Male and Female Comparison.**

Descriptive and ANOVA analysis was performed in SPSS to look for differences or similarities between samples taken from male and female participants. The means, standard deviations and confidence levels can be seen Table 134

Table 134 - Descriptive statistics comparing cation concentrations between male and female participants

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Na^+ conc mmol/L per g mucus	male	34	130.60	16.43	2.82	124.87	136.33	93.99	161.80
	female	27	130.68	12.51	2.41	125.72	135.63	108.39	153.43
	Total	61	130.63	14.71	1.88	126.87	134.40	93.99	161.80
K^+ conc mmol/L per g mucus	male	34	15.12	3.72	0.64	13.83	16.42	9.89	24.68
	female	27	16.73	3.15	0.61	15.48	17.98	10.06	21.98
	Total	61	15.84	3.55	0.45	14.93	16.74	9.89	24.68
Ca^{2+} conc mmol/L per g mucus	male	34	0.93	0.33	0.06	0.81	1.04	0.42	1.65
	female	27	0.85	0.31	0.06	0.73	0.97	0.48	1.78
	Total	61	0.89	0.32	0.04	0.81	0.98	0.42	1.78
Mg^{2+} conc mmol/L per g mucus	male	34	5.20	1.68	0.29	4.61	5.78	2.54	8.94
	female	27	5.20	2.14	0.41	4.35	6.04	2.18	10.90
	Total	61	5.20	1.88	0.24	4.72	5.68	2.18	10.90
Al^{3+} conc mmol/L per g mucus	male	34	0.26	0.34	0.06	0.14	0.38	-0.08	1.30
	female	27	0.28	0.29	0.06	0.16	0.39	-0.01	1.03
	Total	61	0.27	0.32	0.04	0.19	0.35	-0.08	1.30
Cu^{2+} conc mmol/L per g mucus	male	34	0.01	0.00	0.00	0.01	0.01	0.00	0.01
	female	27	0.01	0.00	0.00	0.01	0.01	0.00	0.02
	Total	61	0.01	0.00	0.00	0.01	0.01	0.00	0.02
Fe^{3+} conc mmol/L per g mucus	male	34	0.09	0.05	0.01	0.08	0.11	0.02	0.18
	female	27	0.12	0.07	0.01	0.10	0.15	0.02	0.35
	Total	61	0.11	0.06	0.01	0.09	0.12	0.02	0.35
Mn^{2+} conc mmol/L per g mucus	male	34	0.001	0.002	0.000	0.001	0.002	-0.003	0.004
	female	27	0.002	0.002	0.000	0.001	0.002	-0.001	0.006
	Total	61	0.001	0.002	0.000	0.001	0.002	-0.003	0.006
Zn^{2+} conc mmol/L per g mucus	male	34	0.03	0.02	0.00	0.02	0.03	0.00	0.08
	female	27	0.02	0.01	0.00	0.02	0.03	0.01	0.07
	Total	61	0.03	0.02	0.00	0.02	0.03	0.00	0.08

With ANOVA, the NULL hypothesis assumes that the means are the same. The alterative hypothesis assumes the means are different. If the F value, the ratio of the 2 mean squares, is close to 1 then the NULL hypothesis cannot be rejected. If the value is greater than 1 then the NULL hypothesis can be rejected. The ANOVA details can be seen in **Error! Reference source not found.**

Table 135 -One-way ANOVA between male and female groups

		Sum of Squares	df	Mean Square	F	Sig.
Na^+ conc mmol/L per g mucus	Between Groups	0.083	1	0.083	0	0.985
	Within Groups	12975.777	59	219.928		
	Total	12975.86	60			
K^+ conc mmol/L per g mucus	Between Groups	38.78	1	38.78	3.195	0.079
	Within Groups	716.053	59	12.136		
	Total	754.833	60			
Ca^{2+} conc mmol/L per g mucus	Between Groups	0.09	1	0.09	0.854	0.359
	Within Groups	6.228	59	0.106		
	Total	6.319	60			
Mg^{2+} conc mmol/L per g mucus	Between Groups	0	1	0	0	0.995
	Within Groups	211.875	59	3.591		
	Total	211.876	60			
Al^{3+} conc mmol/L per g mucus	Between Groups	0.004	1	0.004	0.042	0.838
	Within Groups	6.07	59	0.103		
	Total	6.075	60			
Cu^{2+} conc mmol/L per g mucus	Between Groups	0	1	0	0	0.99
	Within Groups	0.001	59	0		
	Total	0.001	60			
Fe^{3+} conc mmol/L per g mucus	Between Groups	0.015	1	0.015	4.177	0.045
	Within Groups	0.218	59	0.004		
	Total	0.233	60			
Mn^{2+} conc mmol/L per g mucus	Between Groups	0	1	0	0.826	0.367
	Within Groups	0	59	0		
	Total	0	60			
Zn^{2+} conc mmol/L per g mucus	Between Groups	0	1	0	0.63	0.43
	Within Groups	0.017	59	0		
	Total	0.017	60			

The NULL hypothesis cannot be rejected for Na^+, Mg^{2+}, Ca^{2+}, Al^{3+}, Cu^{2+}, Mn^{2+} or Zn^{2+}, meaning the means of males and females are statistically the same. The NULL hypothesis can be rejected for K^+ and Fe^{3+} as these are statistically different between the male and female groups. The only significant differences between male and female concentrations are between those for Fe^{3+} and K^+. These differences have not been reported in other published data. The differences between the Fe^{3+} concentrations are small but there is greater Fe^{3+} concentration in nasal mucus from female participants. It is possible that there may have been small spots of blood in samples taken which were not visible to a visual inspection. The differences were not great enough to make

any assumptions that gender had an impact on Fe^{3+} concentration. K^+ levels fluctuate between both genders.

6.3.2 **Analysis of Nasal Mucus: Left and Right Nostril Comparison.**

ANOVA and descriptive analysis were performed to look for similarities between the samples taken from the left and right nasal passages. The descriptive statistics can be seen in Table 136 displaying the mean, standard deviation, and confidence intervals for the concentration values.

Table 136 - Descriptive statistics comparing cation concentrations between the left and right nasal passages

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Na^+ conc mmol/L per g mucus	Left	30	130.44	15.35	2.80	124.70	136.17	93.99	153.43
	Right	31	130.83	14.30	2.57	125.58	136.07	101.89	161.80
	Total	61	130.63	14.71	1.88	126.87	134.40	93.99	161.80
K^+ conc mmol/L per g mucus	Left	30	15.24	3.45	0.63	13.95	16.53	9.89	24.68
	Right	31	16.41	3.60	0.65	15.09	17.73	10.93	22.61
	Total	61	15.84	3.55	0.45	14.93	16.74	9.89	24.68
Ca^{2+} conc mmol/L per g mucus	Left	30	0.92	0.34	0.06	0.79	1.04	0.42	1.61
	Right	31	0.87	0.31	0.06	0.76	0.98	0.47	1.78
	Total	61	0.89	0.32	0.04	0.81	0.98	0.42	1.78
Mg^{2+} conc mmol/L per g mucus	Left	30	5.22	1.84	0.34	4.53	5.91	2.18	10.00
	Right	31	5.18	1.95	0.35	4.46	5.89	2.60	10.90
	Total	61	5.20	1.88	0.24	4.72	5.68	2.18	10.90
Al^{3+} conc mmol/L per g mucus	Left	30	0.27	0.32	0.06	0.16	0.39	-0.01	1.30
	Right	31	0.27	0.33	0.06	0.15	0.39	-0.08	1.22
	Total	61	0.27	0.32	0.04	0.19	0.35	-0.08	1.30
Cu^{2+} conc mmol/L per g mucus	Left	30	0.01	0.00	0.00	0.01	0.01	0.00	0.02
	Right	31	0.01	0.00	0.00	0.01	0.01	0.00	0.01
	Total	61	0.01	0.00	0.00	0.01	0.01	0.00	0.02
Fe^{3+} conc mmol/L per g mucus	Left	30	0.11	0.06	0.01	0.09	0.13	0.02	0.25
	Right	31	0.11	0.07	0.01	0.08	0.13	0.03	0.35
	Total	61	0.11	0.06	0.01	0.09	0.12	0.02	0.35
Mn^{2+} conc mmol/L per g mucus	Left	30	0.00	0.00	0.00	0.00	0.00	0.00	0.01
	Right	31	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total	61	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Zn^{2+} conc mmol/L per g mucus	Left	30	0.03	0.02	0.00	0.02	0.03	0.00	0.07
	Right	31	0.03	0.02	0.00	0.02	0.03	0.01	0.08
	Total	61	0.03	0.02	0.00	0.02	0.03	0.00	0.08

The ANOVA results can be seen in Table 137. The NULL hypothesis cannot be rejected for Na^+, Mg^{2+}, Ca^{2+}, Al^{3+}, Cu^{2+}, Fe^{3+}, Mn^{2+} or Zn^{2+}. They all have an F value of less than 1, indicating that the means are statistically the same. K^+ has an F value greater than 1, meaning the means are statistically different. K^+ values fluctuate. K^+ is an intercellular cation and the exfoliation of dead cells into nasal mucus may account for the fluctuation.

Table 137 - ANOVA between left and right nostril groups

		Sum of Squares	df	Mean Square	F	Sig.
Na^+ conc mmol/L per g mucus	Between Groups	2.324	1	2.324	0.011	0.918
	Within Groups	12973.536	59	219.89		
	Total	12975.86	60			
K^+ conc mmol/L per g mucus	Between Groups	20.903	1	20.903	1.68	0.2
	Within Groups	733.93	59	12.439		
	Total	754.833	60			
Ca^{2+} conc mmol/L per g mucus	Between Groups	0.035	1	0.035	0.327	0.57
	Within Groups	6.284	59	0.107		
	Total	6.319	60			
Mg^{2+} conc mmol/L per g mucus	Between Groups	0.031	1	0.031	0.009	0.926
	Within Groups	211.844	59	3.591		
	Total	211.876	60			
Al^{3+} conc mmol/L per g mucus	Between Groups	0.001	1	0.001	0.01	0.919
	Within Groups	6.074	59	0.103		
	Total	6.075	60			
Cu^{2+} conc mmol/L per g mucus	Between Groups	0	1	0	0.395	0.532
	Within Groups	0.001	59	0		
	Total	0.001	60			
Fe^{3+} conc mmol/L per g mucus	Between Groups	0	1	0	0.001	0.971
	Within Groups	0.233	59	0.004		
	Total	0.233	60			
Mn^{2+} conc mmol/L per g mucus	Between Groups	0	1	0	0.05	0.824
	Within Groups	0	59	0		
	Total	0	60			
Zn^{2+} conc mmol/L per g mucus	Between Groups	0	1	0	0.011	0.915
	Within Groups	0.017	59	0		
	Total	0.017	60			

A correlation report was generated in SPSS to look for any correlations between the cations in nasal mucus. The correlations can be seen in Table 138. The greatest correlation is between Mg^{2+} and Ca^{2+}. Al^{3+} shows a slight correlation with Zn^{2+} and Mn^{2+}. It is not unexpected that Al^{3+} does not correlate highly with any of the bulk metals as it has no biological function in the human body. Mg^{2+} correlates with all but Al^{3+} in the trace elements. Zn^{2+} correlates highly with all but Al^{3+}. Na^+ and K^+ have little correlation with the other cations. The results obtained give consistent results against a reasonably large subject group of students. These results give a good baseline to investigate nasal mucus further looking at differing age groups, and groups of patients with differing nasal conditions

Table 138 - Correlations between the cations

		Na^{+} conc mmol/L per g mucus	K^{+} conc mmol/L per g mucus	Mg^{2+} conc mg/L per g mucus	Ca^{2+} conc mg/L per g mucus	Al^{3+} Sample mg/L per g mucus	Cu^{2+} Sample mg/L per g mucus	Fe^{3+} Sample mg/L per g mucus	Mn^{2+} Sample mg/L per g mucus	Zn^{2+} Sample mg/L per g mucus
Na^{+} conc mmol/L per g mucus	Pearson Correlation	1	-0.215	0.247	.472**	-0.055	0.051	0.122	0.148	.418**
	Sig. (2-tailed)		0.096	0.055	0.000	0.673	0.699	0.350	0.254	0.001
K^{+} conc mmol/L per g mucus	Pearson Correlation	-0.215	1	-0.093	-0.021	0.075	-.312*	-0.115	0.035	-.337**
	Sig. (2-tailed)	0.096		0.476	0.872	0.565	0.014	0.377	0.790	0.008
Mg^{2+} conc mg/L per g mucus	Pearson Correlation	0.247	-0.093	1	.696**	0.013	.510**	.402**	.347**	.620**
	Sig. (2-tailed)	0.055	0.476		0.000	0.922	0.000	0.001	0.006	0.000
Ca^{2+} conc mmol/L per g mucus	Pearson Correlation	.472**	-0.021	.696**	1	0.122	0.210	.256*	.320*	.569**
	Sig. (2-tailed)	0.000	0.872	0.000		0.350	0.104	0.046	0.012	0.000
Al^{3+} Sample mg/L per g mucus	Pearson Correlation	-0.055	0.075	0.013	0.122	1	0.216	0.199	.331**	.286*
	Sig. (2-tailed)	0.673	0.565	0.922	0.350		0.095	0.124	0.009	0.025
Cu^{2+} Sample mg/L per g mucus	Pearson Correlation	0.051	-.312*	.510**	0.210	0.216	1	.461**	.463**	.493**
	Sig. (2-tailed)	0.699	0.014	0.000	0.104	0.095		0.000	0.000	0.000
Fe^{3+} Sample mg/L per g mucus	Pearson Correlation	0.122	-0.115	.402**	.256*	0.199	.461**	1	.470**	.445**
	Sig. (2-tailed)	0.350	0.377	0.001	0.046	0.124	0.000		0.000	0.000
Mn^{2+} Sample mg/L per g mucus	Pearson Correlation	0.148	0.035	.347**	.320*	.331**	.463**	.470**	1	.388**
	Sig. (2-tailed)	0.254	0.790	0.006	0.012	0.009	0.000	0.000		0.002
Zn^{2+} Sample mg/L per g mucus	Pearson Correlation	.418**	-.337**	.620**	.569**	.286*	.493**	.445**	.388**	1
	Sig. (2-tailed)	0.001	0.008	0.000	0.000	0.025	0.000	0.000	0.002	

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

6.4 Experimental

6.4.1 Nasal Mucus Analysis

All standards were pre-prepared by the lab technician in the Department of Geography and Earth Sciences. The Agilent 7700 ICP-MS was calibrated from 0 – 200 ng/mL for 50+ elements and trace metals, with a ruthenium standard. The remaining 80 samples, ID's 120015 – 120094) suspended on cotton wool, 40 pairs, from 20 female and 20 male participants were digested in 2 mL 68% nitric acid. Samples were digested over a 4 h time period, for 1 h at 80 °C, 1 hoat 100 °C and a further 2 h at 120 °C. The digested samples, with the cotton wool splint, were added to a 10 mL volumetric flask and ultra-pure water added to make up to 10 mL. This solution was filtered using an 0.2-micron pipette filter. Between 7 and 9 mL of the solution was recovered. To obtain a representative value for the cations present in the cotton wool splint, 20 control samples were prepared. It is not possible to get an accurate value for the cations in the cotton wool, but by analysing 20 control samples a good approximation can be made. Cotton wool splints of approximately 120 mg were also digested in 2 mL 68% nitric acid and followed the same process as above. The samples were analysed via an Agilent 7700 ICP-MS for the presence of Cu^{+}, Fe^{2+}, Al^{3+}, Mn^{2+} and Zn^{2+} and via Spectra AAS for Na^{+}, K^{+}, Mg^{2+} and Ca^{2+}. The trace metal elements were analysed via ICP-MS at Aberystwyth University. All standards were prepared by their laboratory technician. The ICP-MS was calibrated from 0 – 200 ng/mL for 50+ elements and trace metals, with a ruthenium standard. Calibration curves for the 5 trace metals of interest can be seen in Appendix N.

6.4.2 Experimental AAS

All chemicals were purchased from Sigma Aldrich or Fisher Scientific and were used as directed

The bulk elements Na^{+}, K^{+}, Mg^{2+} and Ca^{2+} were analysed by AAS. Prior to analysis, the 20 cotton wool samples were diluted in ultra-pure water, 1 mL of sample in 10 mL for K^{+}, Mg^{2+} and Ca^{2+} and 0.1 mL of sample in 10 mL of water for Na^{+}.

Calibration standards were prepared from TraceCERT®, 1000 mg/L Na^+ in HNO_3 (1M), 1000 mg/L K^+ in HNO_3 (1M), 1000 mg/L Mg^{2+} in HNO_3 (1M), 1000 mg/L Ca^{2+} in HNO_3 (1M). Calibration standards were prepared for the 4 elements. The calibration standards were prepared in the ranges in Table 139.

Table 139 - Calibration standards for the 4 bulk metals in nasal mucus

Element		Std1 (mg/L)	Std2 (mg/L)	Std3 (mg/L)	Std4 (mg/L)	Std5 (mg/L)	Std 6(mg/L)	Std7(mg/L)
Na^+	Blank	0.25	0.5	0.75	1	1.5	1.75	2
K^+	Blank	1	2	3	3.5	4	5	6
Mg^{2+}	Blank	0.15	0.2	0.25	0.3	0.4	0.5	1
Ca^{2+}	Blank	0.25	0.5	1	1.5	2	2.5	3

7 Conclusions and Future Work

During this study there were several findings made.

Many analytical techniques were used during the analysis of the nasal sprays and nasal mucus. These can be seen in Table 140.

Table 140 - Analytical techniques used during this study

	Nasal Sprays		Nasal Mucus	
Analytical Technique	Bulk Metals	Trace Metals	Bulk Metals	Trace Metals
IC	Y	N	N	N
ICP-OES	Y	Y	N	N
ICP-MS	N	Y	N	Y
AAS	Y	N	Y	N

IC was initially used in the analysis of the nasal wash products, but there were problems resolving peaks for Mg^{2+} and Ca^{2+}. As the samples were acidified during digestion which rendered the samples unsuitable for analysis *via* IC. This led to AAS being used for bulk metals. AAS was not used for trace metals as the samples fell below the LoD for Cu^{2+}, Fe^{3+}, Zn^{2+} and Mn^{2+}. ICP-MS was chosen for trace metals.

Osmolality and pH measurements were taken for nasal wash samples. It was not possible to analyse nasal mucus for a pH value. All nasal mucus samples were suspended on cotton wool; therefore, it was not possible to obtain a pH value or an osmolality reading.

The study found that seawater-based products were predominantly diluted seawater and through analysis *via* ICP-MS and AAS it was possible to see where products had been enhanced with additional minerals. The study looked at a comparison of the cation composition of nasal mucus, in relation to the Na^{+} concentration. The mean concentration in nasal mucus was approximately 130 mmol/L. The values for each of the products was factored to 130 mmol/L to allow for a direct comparison. These values can be seen in Table 141.

Table 141 - Comparison between cation concentrations in nasal mucus, 6 commercial products and seawater with the products. Factored to 130 mmol/L with respect to Na^+.

	Na^+ mmol/L	K^+ mmol/L	Mg^{2+} mmol/L	Ca^{2+} mmol/L	Al^{3+} mmol/L	Fe^{3+} mmol/L	Cu^{2+} mmol/L	Zn^{2+} mmol/L	Mn^{2+} mmol/L
Sterimar Stop & Protect Allergy Response	130	2.9	11.9	9.0	0.002	<0.000	0.0002	0.0002	0.00003
Sterimar Cold Defence	130	2.9	12.4	3.8	<0.000	<0.000	0.0001	0.0014	0.00019
Sterimar Nasal Hygiene	130	3.0	11.9	3.4	0.002	<0.000	0.0001	0.0009	0.00007
Sinomarin Hypertonique	130	2.6	11.0	2.9	<0.000	<0.000	0.0001	0.0004	0.00003
Sterimar Congestion Relief	130	2.9	12.3	3.1	<0.000	<0.000	0.0026	0.0004	0.00004
Sterimar Stop & Protect Cold & Sinus Relief	130	3.0	12.4	2.9	0.001	<0.000	0.0050	0.0003	0.00004
Seawater	130	3.3	14.2	3.2	<0.000	<0.000	0.0001	0.0002	0.000004
MUCUS (STUDENTS)	**130**	**15.84**	**0.89**	**5.2**	**0.27**	**0.107**	**0.007**	**0.026**	**0.001**

These findings indicate that it is possible to manufacture a basic daily isotonic or a hypertonic solution with diluted seawater.

To produce a new product, Halen Môn salt products was an option, but due to the washing of the salt during the production process, there was a reduction in the sea minerals present. A decision was made to move forward looking at a product produced from diluted seawater.

Literature was interrogated over the best delivery system for such a product. The literature findings were inconclusive over the best delivery system for such a spray, but the research does indicate that pressure and speed of actuation, coupled with angle of actuation and plume angle give the greatest deposition within the nasal cavity.[47] Either pump action or aerosol delivery systems could deliver the product effectively within the nasal cavity and would need to be trialled.

Using a cotton wool pellet was found to be a reliable and repeatable method for collecting nasal mucus from the adult population. The acceptability of the method was validated *via* a questionnaire and VAS pain score. The size of the cotton wool pellet did not cause undue discomfort and was found to absorb an acceptable yield of mucus for analysis.

Samples were collected in the same room under the same conditions. All samples were collected during the winter months, so summer allergies were not a factor in the volume of mucus produced. The technique gave a yield great enough to be able to analyse the samples for trace metals, something that had previously been under-researched. Bulk metal concentration had previously been investigated, on a small scale, but investigation into trace metal cation concentrations had been limited.

Having the mucus samples suspended on cotton wool presented some challenges for analysis. Solubilising the mucus with DDH_2O was not possible and a destructive method using 68% HNO_3 was used. This proved to be effective in providing a mean cation value that could be attributed to the cotton wool pellet.

Figure 69 gives a visual breakdown of the mean cation concentration found in nasal mucus. This study concentrated on 4 bulk metal cations and 5 trace metal cations.

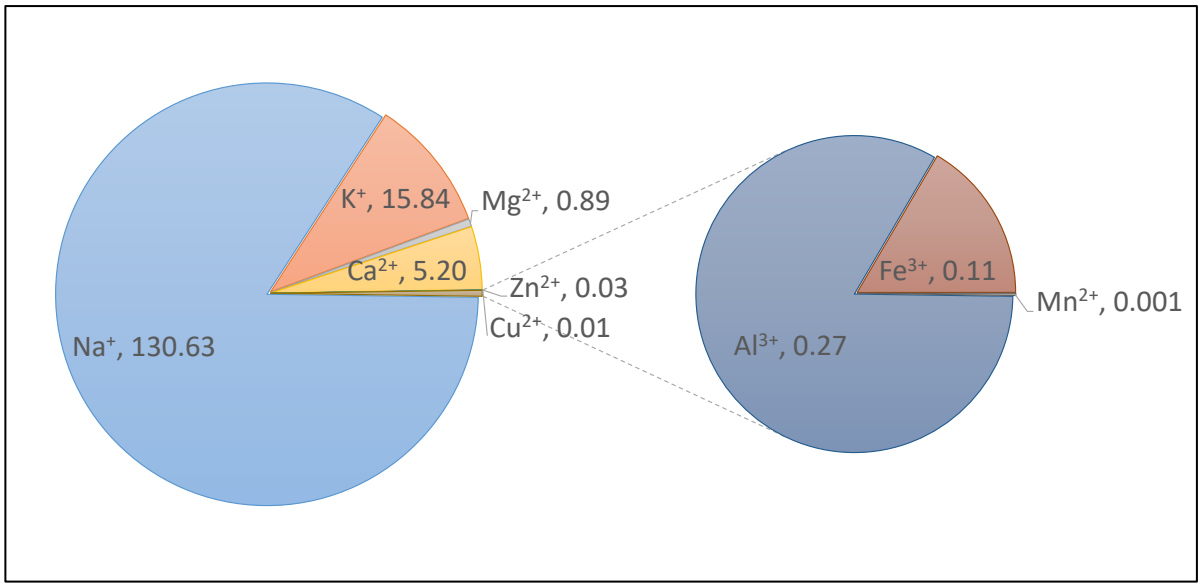


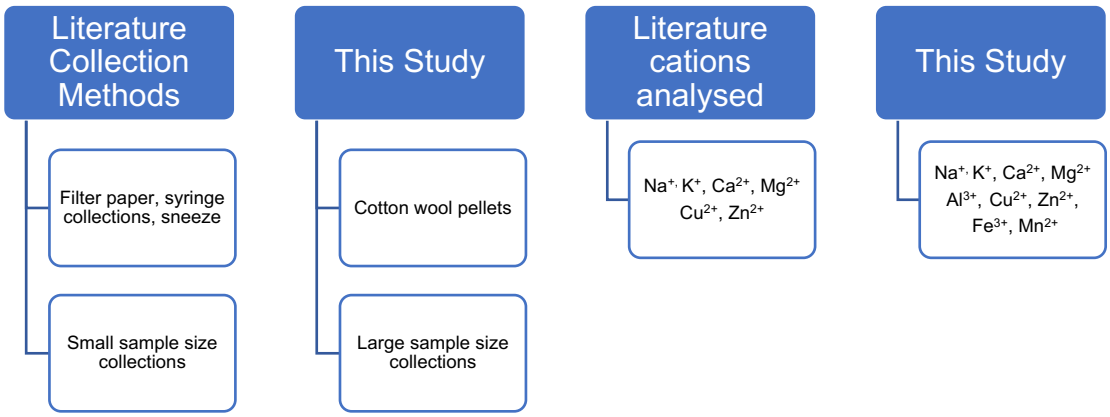
Figure 69 - Visual breakdown of mean concentrations of cations in nasal mucus. All values are in mmol/L

There were many different collection methods and the cations analysed in literature studies differed. The collection methods used, and the cations analysed can be seen in Figure 70.

This study used a larger number of participants than previous ones. Table 142 shows the number of participants and the cation concentrations found in previous studies and

presents a comparison to the results of this study. This study analysed 61 samples, considerably more than other studies. There were 19 in study C[7] with 18 in study G[9] and lower participants in the other studies.

Figure 70 - Comparison of collection methods and cations analysed in literature compared to this study



Previous mucus analysis studies looked at only 4 cations, with these being mainly the bulk metals Na^+, K^+, Mg^{2+} and Ca^{2+}. This study looked at 9, a far greater number.

Table 142 - Comparison table showing the results and participants for literature studies with a comparison to the results of this study

Ion	Study A	Study B	Study C	Study D	Study E	Study F	Study G	Study H	This study
	mM (Mean + SD)	mM (Mean + SD)	mM (Mean + SD)	mM (Mean + SD)	mM - (approx Value)	mM (Mean + SEM)	mM (Mean + SD)*	mM (Mean + SD)**	mM (Mean + SD)
Na^+	127 ± 6	142 ± 28	141 ± 8	150 ± 32	110	184 ± 37		74.5 ± 36.2	130 ± 15
K^+	27 ± 3	43 ± 10	61 ± 8	41 ± 18	30	32.6 ± 5.2		14.4 ±7	16 ± 4
Ca^{2+}	5 ± 1			4 ± 2		1.5 ± 0.27	1.33 ± 0.08	2.2 ± 3.1	5 ± 2
Mg^{2+}						0.72 ± 0.21	0.65± 0.08	0.41 ± 1.03	0.9 ± 0.3
Zn^{2+}							0.002		0.03 ± 0.02
Cu^{2+}							0.002		0.01 ± 0.00
Al^{3+}									0.027 ± 0.32
Mn^{2+}									0.001 ± 0.002
Fe^{3+}									0.11 ± 0.06
No of healthy Participants	8	8	19	10	8	2	7	14	61
No of Pathalogical Participants	3	3	0	0	9	0	11	0	0

A[8] = Vanthnaouvong & Roomins (2004) - Collected with a micropipette
B[8] = Vanthnaouvong & Roomins (2004) - Collected with a Sephadex G-25 ion exchange beads, mounted on tape, applied to filter paper
C[7] = Vanthnaouvong & Roomins (2006) - Collected with a Sephadex G-25 ion exchange beads, mounted on tape, applied to filter paper (results only for healthy participants)
D[4] = Lorin *et al* (2004) Using Filter Paper
E[5] = Knowles *et al* (2000)
F[10] = Burke (2004) - using spontaneous collection method
G[9] = Henkin *et al* (2000)* not for Zinc or copper
H[21] = Narkowicz *et al* (2013)** Results for non-smokers only

Statistical analysis of the data enabled comparisons of trace and bulk cation concentrations of nasal mucus between left/right nostrils, and male/female groups. The data was scrutinised for any correlations between the various cations. This type of data had not been collected in other studies. Data between left and right nostril collections was statistically similar. Data from the comparison of male and female participants found interesting differences in 2 cations, K^+ and Fe^{3+}, as seen in Table 143.

Table 143 - The 2 cations showing statistically the greatest variability in the analysis of nasal mucus between male and female participants

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Fe^{3+} conc mmol/L per g mucus	male	34	0.09	0.05	0.01	0.08	0.11	0.02	0.18
	female	27	0.12	0.07	0.01	0.10	0.15	0.02	0.35
	Total	61	0.11	0.06	0.01	0.09	0.12	0.02	0.35
K^+ conc mmol/L per g mucus	male	34	15.12	3.72	0.64	13.83	16.42	9.89	24.68
	female	27	16.73	3.15	0.61	15.48	17.98	10.06	21.98
	Total	61	15.84	3.55	0.45	14.93	16.74	9.89	24.68

Fe^{3+} levels were higher in female participants. There could be various reasons for this with age and stage within the menstrual cycle being a factor. Medications could also impact the cations present in a person's mucus. Medications for blood pressure could influence the levels of K^+ in the mucus, as could other undiagnosed conditions.

Ages and ethnicities for the participants were collected but were not able to be used in the study. The study used a student population as participants, and as there were only small numbers of older or specific ethnicities, it would have been easy to de-anonymise participants *via* these 2 categories.

Al^{3+} cations were found in all nasal mucus samples. Finding Al^{3+} in nasal mucus has not been expected as aluminium has no biological function in the human body. Al^{3+} was detected in low levels in the cotton wool control samples, but the values detected in the mucus samples was considerably higher. The values for Al^{3+} can be seen in Table 144.

Table 144 - Al^{3}+ cation concentrations found in nasal mucus samples.

		Total			Male			Female		
		N	Mean	Std. Deviation	N	Mean	Std. Deviation	N	Mean	Std. Deviation
Al Sample mmol/L per mg mucus	Left	30	0.274	0.32	17	0.278	0.333	13	0.269	0.304
	Right	31	0.266	0.33	17	0.247	0.363	14	0.289	0.287
	Total	61	0.270	0.32	34	0.262	0.343	27	0.279	0.290

The mean values are approximately 0.28 mmol/L per mg of mucus, but with a high standard deviation. It is unclear where the Al^{3+} originates, but it is likely to be environmental. It may be being breathed in from the atmosphere or linked to spray deodorant particles inhaled, taken in through the skin through stick deodorants or inhaled through vaping. This area warrants further investigation.

Additional studies could be performed on looking at Al^{3+} content in spray deodorants per actuation across differing brands. The type and brand of deodorant used could be a question asked on the questionnaires to tie in with this data.

Participants were not asked if they used vaping products. This could also be asked on the questionnaire as a “Do you use vaping products?” and a question on the frequency of use. Analysis of vaping products could also run alongside a further nasal mucus study.

The questions asked to participants in this study were regarding general health and were extremely brief. Future questionnaires could have more detailed questions to give a deeper understanding into the participants health and lifestyle.

This study had a greater number of participants than other studies, but a larger study group would allow for greater analysis looking at age, smoking or vaping habits, ethnicity, menstrual cycle (female), medications, where participants live, allergies etc. alongside the left/right and male /female groupings to date. The data obtained to date gives a good foundation to move this research on further.

Adjustment to collection and analysis of the cotton wool pellets could be improved. In this study cotton wool control samples were prepared as pellets of approx. 130 mg in mass and digested using the same volume of HNO_3. Digestion of cotton wool could be performed on a larger scale, e.g., 1 Kg of cotton wool, to give an average cation concentration during analysis.

The pain calculation in this study looked at how the participant perceived the pain of the collection to another life experience. This was very subjective. In a future study this could be compared to the pain experienced during a COVID-19 PCR test, something that became common after the data was collected for this study.

Another necessary step in any future research would be to look at the cation concentrations in the mucus of patients with a history of nasal condition such as allergic rhinitis and nasal polyps and compare with a control group with no history of such conditions. Ethical approval has been obtained (IRAS 269189) to carry out this further study. This approval had been obtained to compare nasal mucus cations in a control group such as tonsillectomy patients who otherwise were healthy. A more detailed questionnaire had been approved for this, but should this additional study go ahead it would need to be amended to cover additional questions raised in this study. Unfortunately, due to the COVID-19 pandemic it was not possible to recruit and collect patient samples for analysis. An additional study could offer greater insight into the contribution cation concentrations have on nasal health.

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Appendix A Developing a Routine Method for the Analysis of Nasal Mucus

This is the previous trial carried out at Bangor University investigating a new collection method for nasal mucus

A1 – Developing a Routine Method for the Analysis of Nasal Mucus

A2 – Participants' Reactions to Nasal Mucus Collection

A1 – Developing a Routine Method for the Analysis of Nasal Mucus

Developing a Routine Method for the Analysis of Nasal Mucus.

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1 Objectives

If there were tests on nasal mucus, equivalent to the venous blood sample, the knowledge and clinical gain would be significant. No such test is in routine use.

2 Method

Healthy volunteers had nasal mucus passively collected from the nose for 15 minutes without local anaesthesia (Figure 1). On one side an inert plastic splint was used, on the other simple cotton wool. The splint was a standard 42 mm silastic blue nasal splint (Exmoor Plastics), the cotton wool varied in weight from 120 to 300 mg. We measured the yield by weight of mucus on each side. The participant experience is reported in a sister paper.

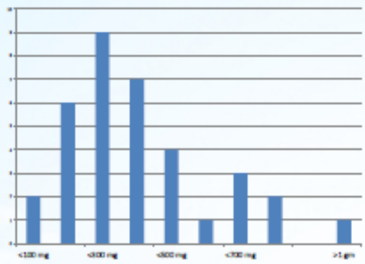
3 Figure 1

Participant During Mucus Collection

4 Results

36 subjects have been tested, of whom all 36 tolerated the cotton wool, but only 32 tolerated the plastic splint. The average yield of mucus onto the splint was 190 mg (SD 110 mg, range 80-460 mg), and onto cotton wool was 500 mg (SD 350 mg, range 110 mg - 1.52 gm).

5 Figure 2



Nasal mucus yield standardised to 100 mg of cotton wool in 15 mins

6 Discussion

The plastic splint tended to be problematic, not only due to pain, but also with mucus failing to adhere easily, and falling off on removal. Standardising for 100mg of cotton wool in 15 minutes gave an average yield of 324 mg (SD 176 mg, range 80 - 1340 mg/15 mins). See Figure 1. The sub-group of subjects with much higher yields, giving a second peak, were apparently those with hay fever/ allergic rhinitis, but were tested out of season. No dedicated allergy testing was undertaken.

The analytic and diagnostic options with nasal mucus will vary with the amount collected, and how dilute it is. Previous approaches have either relied on collection onto paper strips followed by elution (1,2) or collecting saline after irrigation of the nasal cavity (3). Neither is an accurate quantitative method, suitable for diagnostics. These studies also attest to the great variation in nasal mucus production between individuals.

7 Conclusions

Using inert plastic splints to collect native nasal mucus is unlikely to be practical without dedicated design work. The rate of nasal mucus production has at least a 9-fold range. Analysis of nasal mucus chemistry may require a soft, absorbent matrix, and then secondary separation before testing, if it is to become practical and routine.

References

1 Knowles, G. K., P. Townsend, and M. Turner-Warwick. "A standardized filter paper technique for assessing nasal secretory activity." Clinical & Experimental Allergy 11.3 (1981): 287-292.
2 Lorin, M. I., P. F. Gaerlan, and I. D. Mandel. "Quantitative composition of nasal secretions in normal subjects." The Journal of Laboratory and Clinical Medicine 80.2 (1972): 275-281.
3 Swart, Sieke J., et al. "Immunoglobulin concentrations in nasal secretions differ between patients with an IgE-mediated rhinopathy and a non-IgE-mediated rhinopathy." Journal of Allergy and Clinical Immunology 88.4 (1991): 612-619.

A2 – Participants' Reactions to Nasal Mucus Collection

Participants' Reactions to Nasal Mucus Collection

David Hill[1], Loretta Murphy[2] and Charles Ben-Cofie[2]
[1]Ysbyty Gwynedd, Bangor, Gwynedd, [2]School of Chemistry, Bangor University, Gwynedd.

GIG CYMRU NHS WALES | Bwrdd Iechyd Prifysgol Betsi Cadwaladr University Health Board | Awyr Las Blue Sky | Prifysgol BANGOR University | Ysgol Cemeg School of Chemistry

1 Objectives

There are no tests for the qualitative or quantitative aspects of nasal mucus in routine use. Unlike the routine analysis of many other body fluids, such as blood or urine, nasal mucus is an unknown. CSF, pancreatic secretions, and even ovarian follicle fluid are easier to collect and analyse.

2 Method

Healthy volunteers had nasal mucus passively collected from the nose for 15 minutes without local anaesthesia (Figure 1). On one side an inert plastic splint was used, on the other simple cotton wool. The cotton wool was a 40 mm length of twisted cotton wool, the splint was 43 mm long. Here we report the participant experience, and speculate on what may be an acceptable level of discomfort for the yield of mucus. The yield on each side is reported in a sister paper.

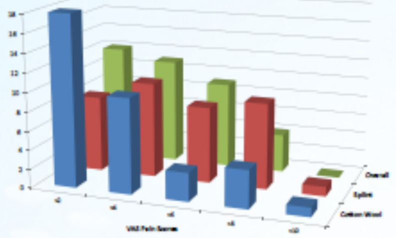
3 Figure 1

Participant During Mucus Collection

4 Results

36 subjects have been tested, of whom all 36 tolerated the cotton wool, but only 32 tolerated the plastic splint. Adverse events for the splints included one refusal, two spontaneous expulsions, and one posterior displacement. In a binary questionnaire, pain was reported with 21/32 splints, and 12/36 cotton wool insertions.
Average pain scores on a VAS were 4.13 for splints (SD 2.56, range 0.4 – 7.2), and 2.6 for cotton wool (SD 2.1, range 0.0 – 8.3). The distribution of VAS scores is shown in Figure 2.

5 Figure 2



Nasal Sampling Acceptability VAS Scores - (No Local An.)

6 Discussion

The data presented in Figure 2 clearly shows the cotton wool was less painful, but it is interesting that even in this group approximately 20% of subjects found the experience significantly painful. This may be a limiting factor in routine collection of mucus, just as the phenomenon of needle phobia is a limiting factor in blood collection. The prevalence of needle phobia has been estimated at 2% in a travellers' health clinic and 10% in the general population (refs 1, 2)
Transient feelings of faintness were recorded by 2/36 subjects. In a free text section ("Use three words"), the commonest terms used were uncomfortable (18), unpleasant (5), unusual (5), painful (4), and interesting (4). The word 'gross' was used twice, and the word 'odd' only once.
Related studies have shown that the use of local anaesthesia spray has no net benefit in the context of OPD nasendoscopy (Refs 3, 4) when one measures pain, bad taste, and overall discomfort. We therefore decided to avoid the use of these agents, which could affect the yield and composition of mucus. We will continue to refine the technique of native, undiluted nasal mucus collection in the hope that we can collect this on a routine basis.

7 Conclusions

Using inert plastic splints to collect native nasal mucus is unlikely to be practical without further design work, but cotton wool is broadly acceptable. Normal individuals can tolerate collection without anaesthesia, but most will find it moderately unpleasant. A group of about 20% will still find collection with cotton wool difficult.

References
1 Nir Y, Paz A, Sabo E, Potasman I. Fear of injections in young adults: prevalence and associations. Am J Trop Med Hyg 2003;68:341-4.
2 Hamilton JG. Needle phobia: a neglected diagnosis. J Fam Pract 1995;41:169-75.
3. A.J. Cain, D.P. Murray and L.G. McClymont. The use of topical nasal anaesthesia before flexible nasendoscopy: a double-blind, randomized controlled trial comparing cophenylcaine with placebo. Clinical Otolaryngology & Allied Sciences 2002; Vol 27 (6), 485-488.
4. Frosh AC, Jayaraj S, Porter G, Almeyda J. Is local anaesthesia actually beneficial in flexible fibreoptic nasendoscopy? Clinical Otolaryngology and Allied Sciences 1998; 23(3):259-262

Appendix B Mucus Collection Forms

A collection of forms used in the collection process to include:

A general information sheets
The consent forms
The post procedure questionnaire

Analysis of Nasal Mucus in Healthy Adults

Information about the research project

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you.

This project is concerned with developing a simple and convenient method of collecting, and analysing, natural nasal mucus in way that does not involve dilution with saline. It is normal clinical practice to introduce cotton wool into the nose, and in this study, we wish to analyse the mucus that adheres to the cotton wool.

One of our team will go through the information sheet with you and answer any questions you have. Explaining this information sheet to you should take about 10 min, and the collection will take another 15 min. Do feel free to talk to others about the study if you wish.

What is the purpose of the study?

The long-term aim of the project is to discover and describe in detail the inorganic chemistry of normal human nasal mucus. In so doing, we will develop a safe and convenient method(s) for collecting nasal mucus, and create new, medically important, knowledge about common nasal diseases. We are seeking to make the collection of nasal mucus for medical analysis as simple as collecting a blood sample is now. In this stage of the study we will be concerned with the analysis of the mucus that has been collected, and also with the experiences of the participants.

The study is being conducted in The School of Chemistry, Bangor University, in collaboration with an ENT Surgeon from Ysbyty Gwynedd, as part of a PhD programme dedicated to this topic.

Who can take part in this study?

Normal, healthy volunteers who are students and staff at Bangor University, or Ysbyty Gwynedd, are being invited to take part in the research. Most people are suitable to participate. The only exclusion criteria are people with a known bleeding disorder, and those with a cold in the week of the experiment.

If you agree to take part, we will then ask you to sign a consent form. This study is independent of the clinical service of the hospital, and no diagnostic information about your nose will be given to you after the tests. You are free to withdraw at any time, without giving a reason.

What will happen to me if I take part?

If you decide to take part, you will be briefly interviewed face-to-face by one of the researchers, and any questions that you have will be answered. You will then sign a simple consent form and be given a copy of this for your own records. The medical researcher, an ENT Surgeon, will then inspect your nose using a small disposable viewer. He will then insert a small piece of cotton wool in one side of your nose, and then a similar piece into the other side. The pieces will slightly smaller than the size of a little finger and will stay for 15 min. You are free to withdraw from the study at any time, both before, during, and after insertion of the cotton wool.

After sitting quietly reading for 15 min in a warm room, the cotton wool will be removed painlessly from your nose. They will then be weighed and stored in a freezer until undergoing analysis at the university. You will then be asked a few questions about the experience by a neutral observer who is not part of the experimental study group and be free to go home. You will receive £20 in cash to cover your time and expenses.

Will my taking part in the study be kept confidential?

Yes. We will follow good ethical and legal practice, and all information about you will be handled in confidence. The data about your nasal mucus will be wholly anonymous, and not linked to your name in any way. The answers from the questionnaire will contain only your study ID (not your name) and will be stored on a secure computer. Data will be kept securely until after publication of the findings, and then destroyed. Anonymised data (not individual interviews) will be stored in a secure data archive. Some anonymised quotes from the interview by the independent neutral observer may be recorded on paper and may be included in a narrative section in a future publication. These quotations would illustrate the reality of using these techniques for routine clinical investigation of nasal mucus.

What are the possible benefits of taking part?

Whilst there are no immediate benefits to yourself for taking part, this is your chance to help develop a simple investigation that has the potential to help people affected by chronic nasal and lung diseases.

What are the possible disadvantages and risks of taking part?

There is low risk of harm by taking part. There is a 1% risk that you may develop a short-lived nosebleed and will need to sit up and bend your head forward over a bowl while this stops with minimal intervention, such as pinching the end of your nose, or sucking small pieces of ice. There is also a similar small risk that you may feel faint, and there will be a medical couch in the experimental area for those people who wish to lie down for a few minutes. There will be a doctor on hand at all times to advise if there are any such problems. There were no problems with bleeding in an earlier study, although 3% of people felt slightly faint.

Who is organising and funding the research?

The research is being led by Mr David Hill, Consultant ENT Surgeon at Ysbyty Gwynedd, Bangor, working with Dr Lorrie Murphy, Senior Lecturer in The School of Chemistry, Bangor University. It is funded by the Welsh Government in the KESS2 research programme, and there is a dedicated PhD student within the team. It has also been funded by the Awyr Las, the charity which supports research and health care studies in North Wales. If you wish to talk further to someone about the research

please contact Dr Murphy on 01248 382384, or on email at l.m.murphy@bangor.ac.uk. .

Who has reviewed the study?

All research in Bangor University is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and passed by The Research Ethics Committee of the School of Natural Sciences of Bangor University, Application Number 01/23/45/67. It has also been passed by the Wales Research Ethics Committee 5, at Ysbyty Gwynedd Hospital, reference number 11/222/333

If you wish to make a complaint about the study, you can contact

Mrs Gwenan Hine,

Secretary to the Ethics Committee and the Ethical Review Committee

Registrar's Office, Bangor University, Bangor, LL57 2DG

Email: gwenan.hine@bangor.ac.uk

Tel 01248382413

Consent Form - Nasal Mucus Analysis

Name of Project: Analysis Methods 1 – Nasal Mucus

Names of Principal Researchers: Dr Lorrie Murphy and Mr David Hill

Please initial all boxes

1. I confirm that I have read and understand the information sheet dated 23/06/2107 (version 1.2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. Fi
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. Fi
3. I understand that the data will be purely for research purposes, with no diagnostic value. Ta
4. I understand the data will be archived securely and anonymously. Ta
5. I understand that the data will be published at the end of the study, and that completely anonymous quotations from the questionnaire may be used in the report/publication. Ta
6. I agree to take part in the study

Name of Participant	Date	Signature
Name of researcher	Date	Signature

Analysing Nasal Mucus with Cotton Wool Splints

Post Experiment Questionnaire

We are interested in finding out how easy or difficult the collection of nasal mucus is for the participants, and how the experiment can be made more acceptable in future. Please answer the following questions, and write some free text about the experience.

1 Did inserting the cotton wool cause pain? **YES** **NO**

2 Did blocking your nose on both sides cause you distress?

YES **NO**

3 Did you feel faint at any time? **YES** **NO**

4 Would you recommend this study to a friend? **YES** **NO**

Please use three words to describe the experience

Please write free text in the box below if you wish

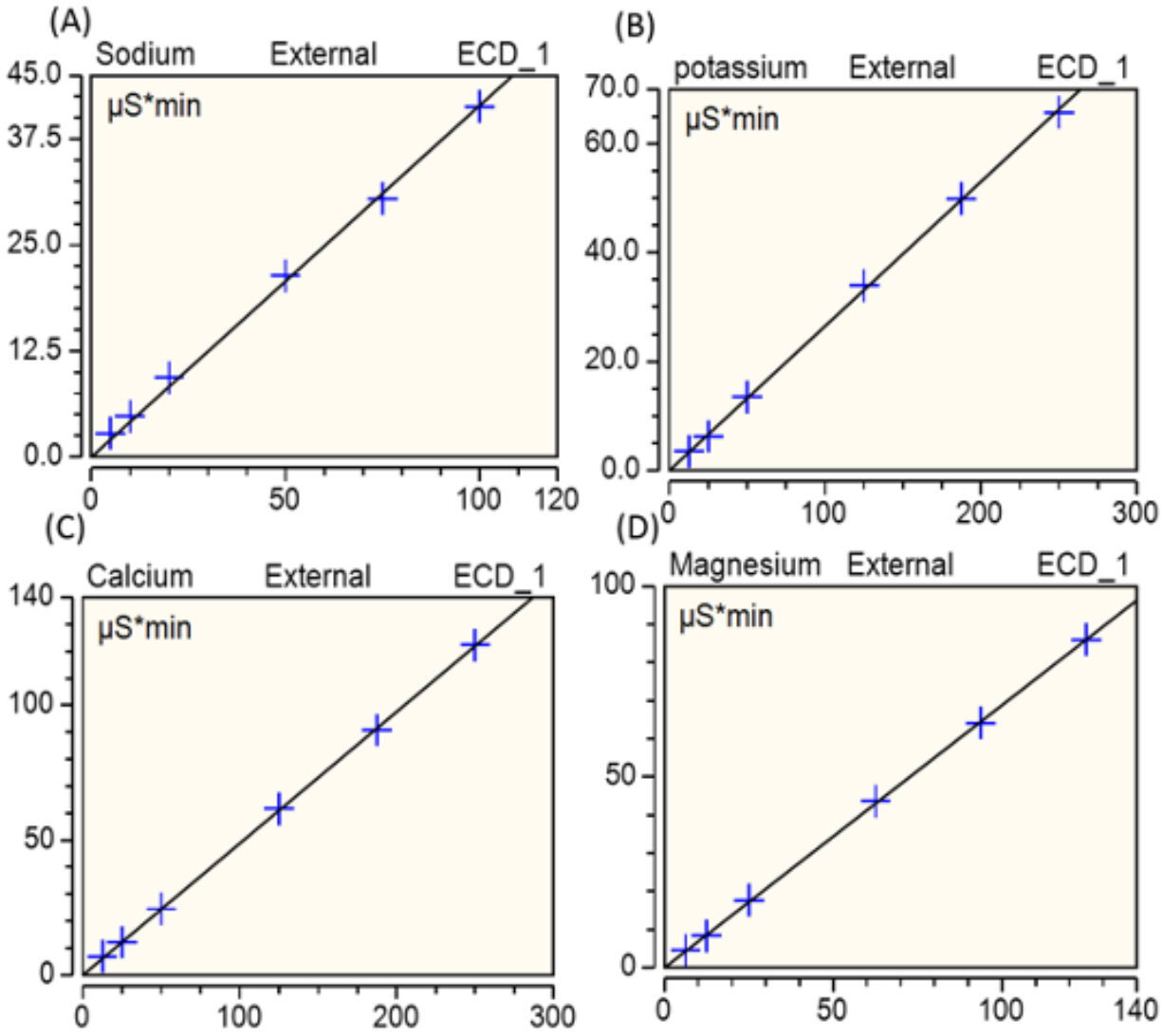
Thank you for your participation in this study – David Hill, Dept of ENT, Ysbyty Gwynedd.

2nd Version – Nasal Mucus Collection Questionnaire 31st May 2017

Appendix C Calibration Curves IC Analysis 2 Nasal Spray Analysis

Calibration curves for (A) Na^+ (B) K^+, (C) Ca^{2+} and (D) Mg^{2+}

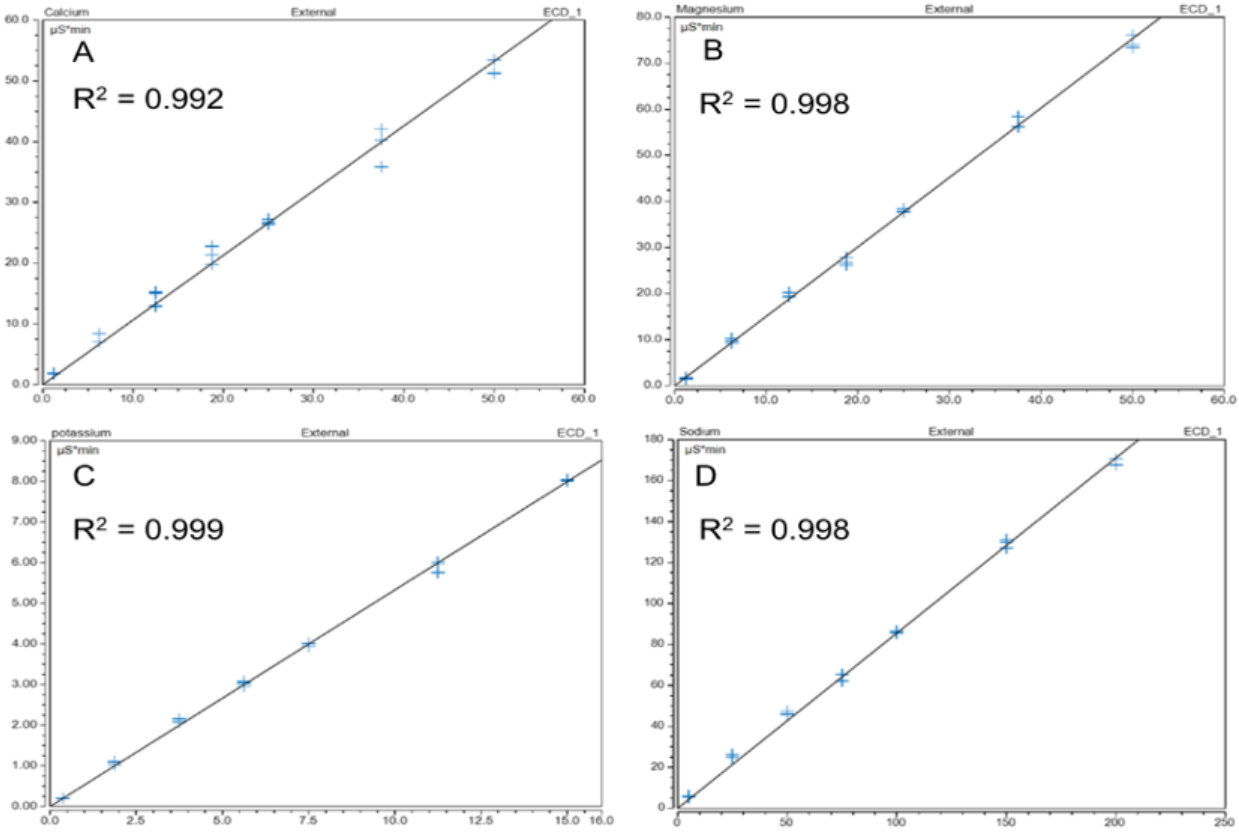
The X-Axis shows the concentration of the calibration standards in mg/L with the Y-Axis showing the area of peak (μs*min)


(A)
Sodium External ECD_1
µS*min
45.0
37.5
25.0
12.5
0.0
0
50
100
120
(B)
potassium External ECD_1
µS*min
70.0
60.0
40.0
20.0
0.0
0
100
200
300
(C)
Calcium External ECD_1
µS*min
140
100
50
0
0
100
200
300
(D)
Magnesium External ECD_1
µS*min
100
50
0
0
50
100
140

Appendix D Calibration Curves IC Analysis 3 Nasal Spray Analysis

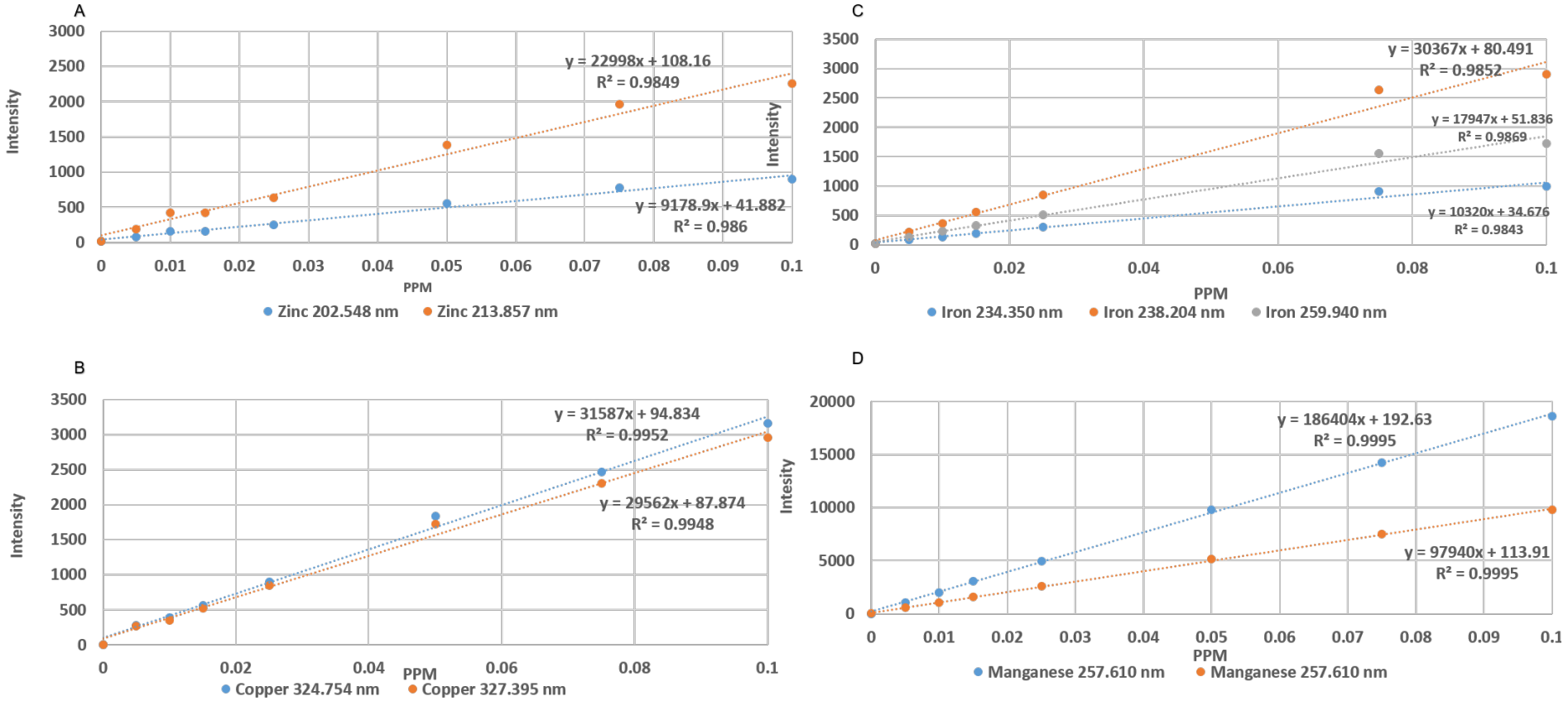
Calibration Curves for, (A) Ca^{2+}, (B) Mg^{2+}, (C) K^{+} and (D) Na^{+}

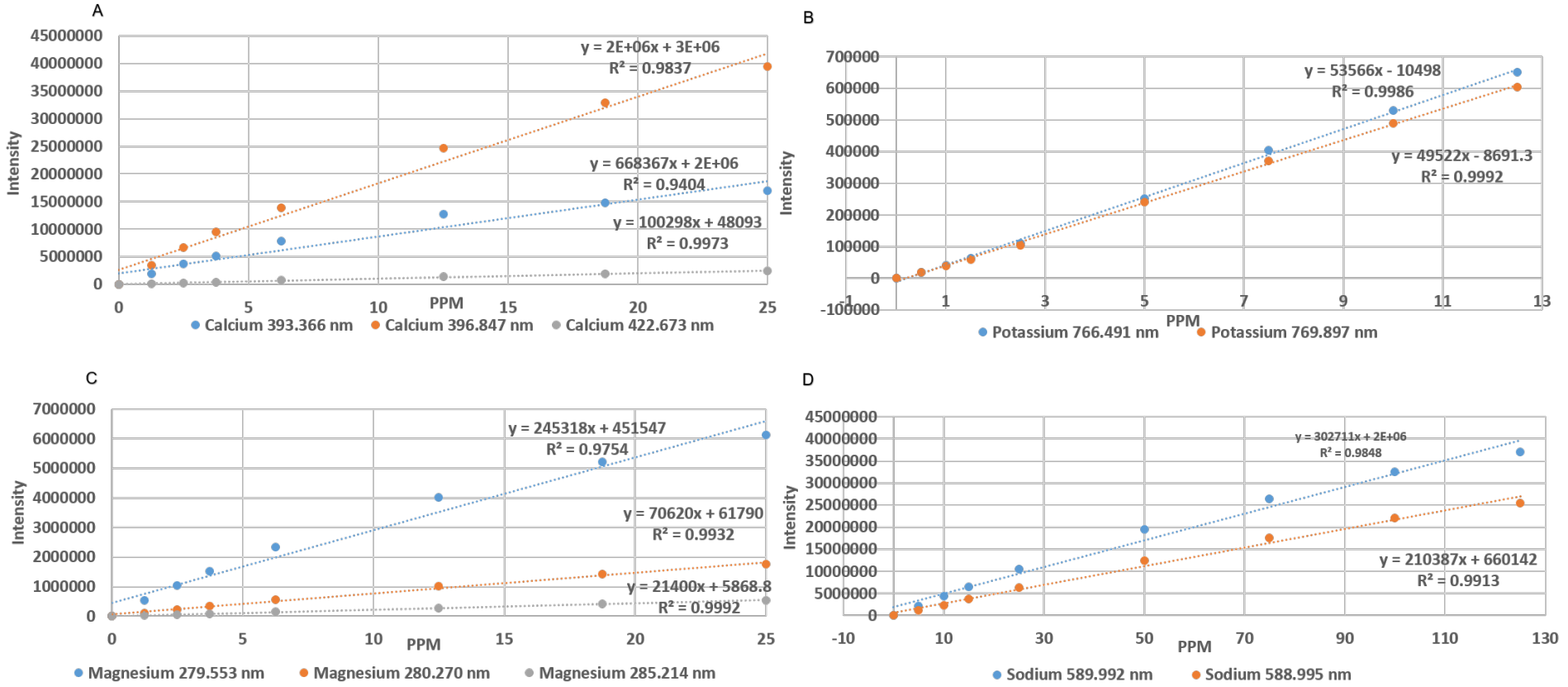
The X-Axis shows the concentration of the calibration standards in mg/L with the Y-Axis showing the area of peak (μs*min)


Calcium
External
ECD_1
µS*min
A
R² = 0.992
Magnesium
External
ECD_1
µS*min
B
R² = 0.998
potassium
External
ECD_1
µS*min
C
R² = 0.999
Sodium
External
ECD_1
µS*min
D
R² = 0.998

Appendix E Calibration Curves ICP-OES 2 Nasal Spray Analysis

Calibration curves for bulk metals Na^{+}, K^{+}, Mg^{2+}, Ca^{2+} and trace metals Zn^{2+}, Cu^{2+}, Fe^{3+} and Mn^{2+}

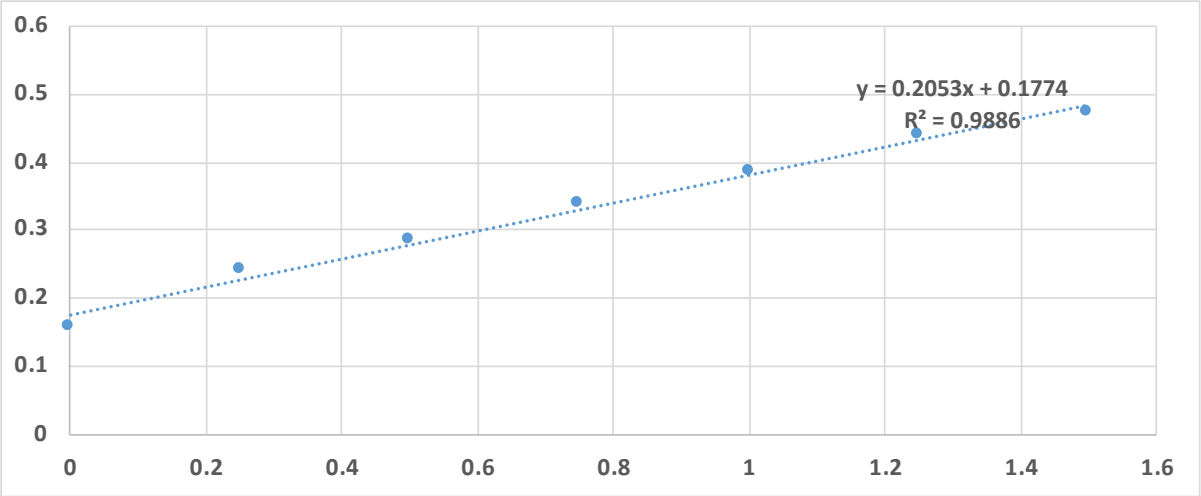

A
Intensity
3000
2500
2000
1500
1000
500
0
y = 22998x + 108.16
R² = 0.9849
y = 9178.9x + 41.882
R² = 0.986
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.1
PPM
Zinc 202.548 nm
Zinc 213.857 nm
C
3500
3000
2500
2000
1500
1000
500
0
y = 30367x + 80.491
R² = 0.9852
y = 17947x + 51.836
R² = 0.9869
y = 10320x + 34.676
R² = 0.9843
0
0.02
0.04
0.06
0.08
0.1
PPM
Iron 234.350 nm
Iron 238.204 nm
Iron 259.940 nm
B
Intensity
3500
3000
2500
2000
1500
1000
500
0
y = 31587x + 94.834
R² = 0.9952
y = 29562x + 87.874
R² = 0.9948
0
0.02
0.04
0.06
0.08
0.1
PPM
Copper 324.754 nm
Copper 327.395 nm
D
Intesity
20000
15000
10000
5000
0
y = 186404x + 192.63
R² = 0.9995
y = 97940x + 113.91
R² = 0.9995
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.1
PPM
Manganese 257.610 nm
Manganese 257.610 nm


A
Intensity
PPM
y = 2E+06x + 3E+06
R² = 0.9837
y = 668367x + 2E+06
R² = 0.9404
y = 100298x + 48093
R² = 0.9973
Calcium 393.366 nm
Calcium 396.847 nm
Calcium 422.673 nm
B
Intensity
PPM
y = 53566x - 10498
R² = 0.9986
y = 49522x - 8691.3
R² = 0.9992
Potassium 766.491 nm
Potassium 769.897 nm
C
Intensity
PPM
y = 245318x + 451547
R² = 0.9754
y = 70620x + 61790
R² = 0.9932
y = 21400x + 5868.8
R² = 0.9992
Magnesium 279.553 nm
Magnesium 280.270 nm
Magnesium 285.214 nm
D
Intensity
PPM
y = 302711x + 2E+06
R² = 0.9848
y = 210387x + 660142
R² = 0.9913
Sodium 589.992 nm
Sodium 588.995 nm

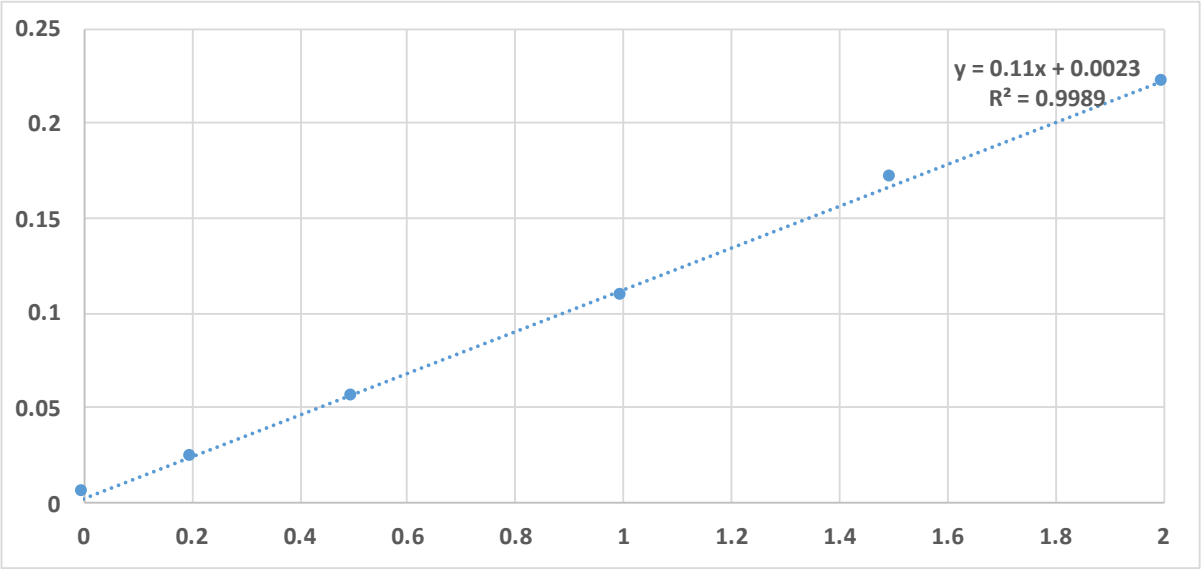
Appendix F AAS Calibration Curves and Calibration Standards Nasal Spray Analysis

Calibration curves for Na^+ (A), K^+ (B), Ca^{2+} (C) and Mg^{2+} (D). All concentrations are in mg/L

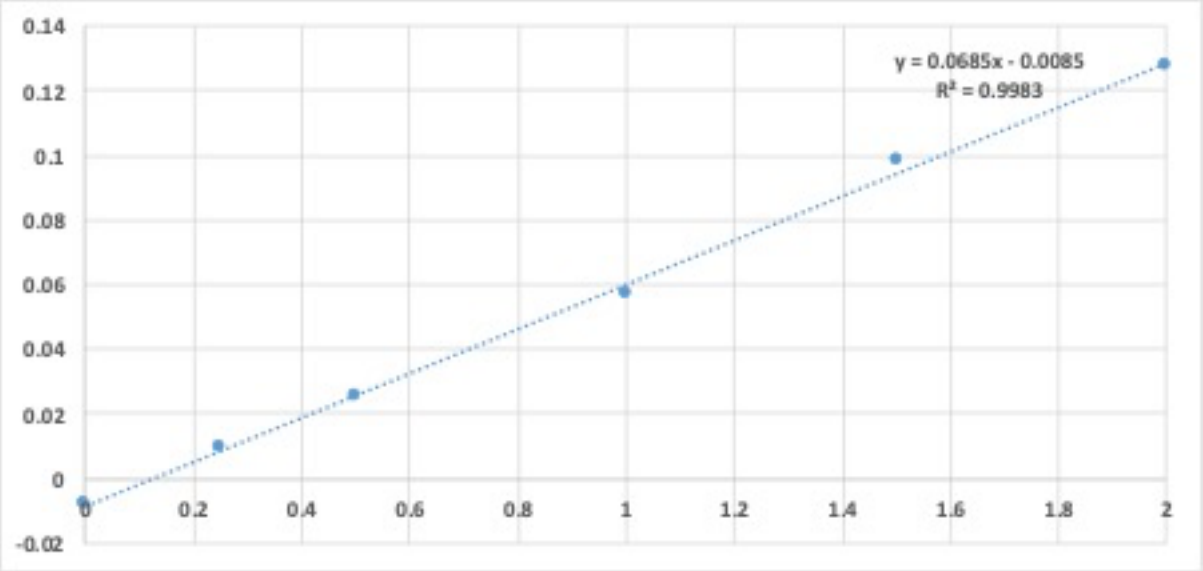
(A) Na^+


y = 0.2053x + 0.1774
R² = 0.9886
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6

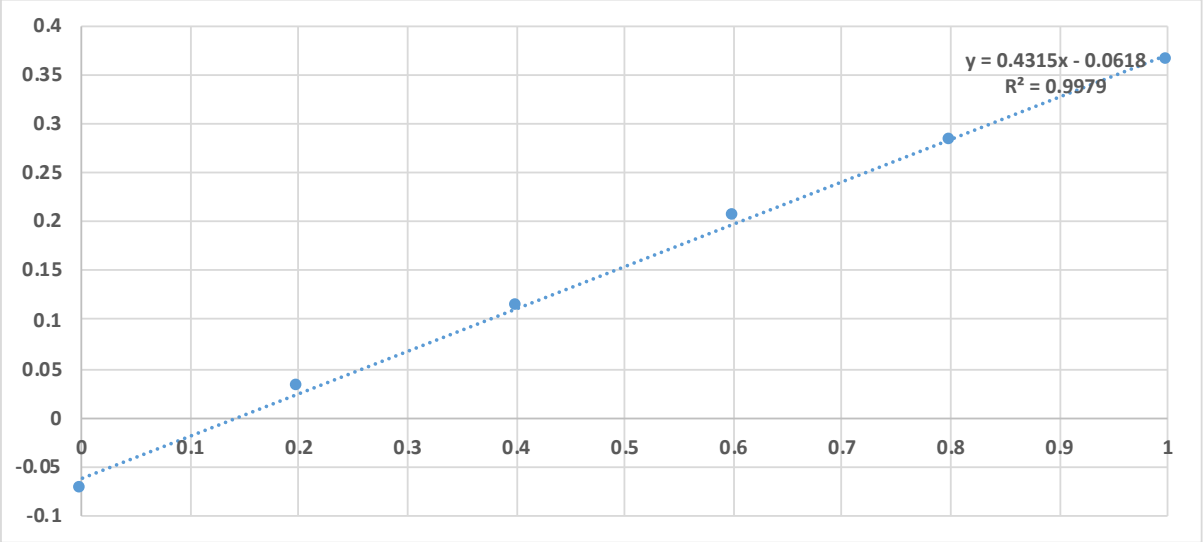
(B) K^+


y = 0.11x + 0.0023
R² = 0.9989
0.25
0.2
0.15
0.1
0.05
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2

(C) Ca^{2+}


y = 0.0685x - 0.0085
R² = 0.9983
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2

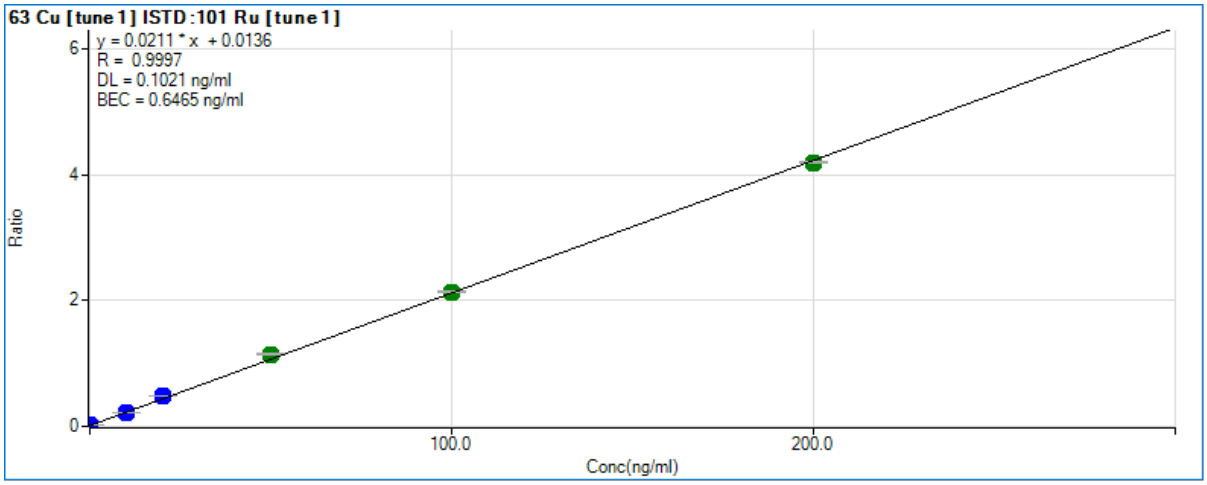
(D) Mg^{2+}


y = 0.4315x - 0.0618
R² = 0.9979
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

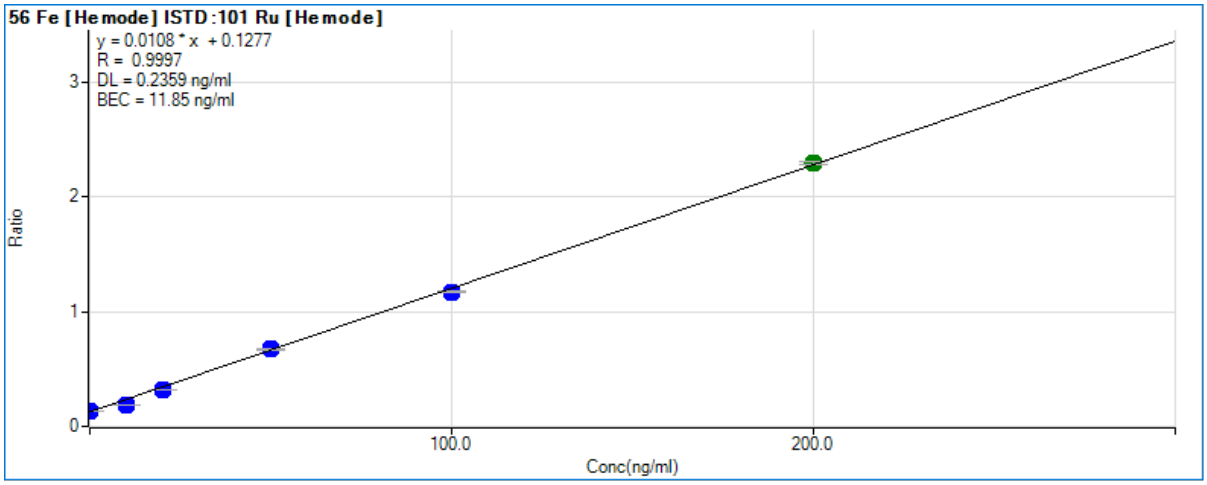
Appendix G Calibration Curves for ICP-MS Analysis 1 Nasal Spray Analysis

Calibration curves for Cu^{2+}(A), Fe^{3+} (B), Mn^{2+} (C) and Zn^{2+} (D)
The error bars indicate the % relative standard deviation calculated during the automated calibration process using Agilent Mass Hunter software.

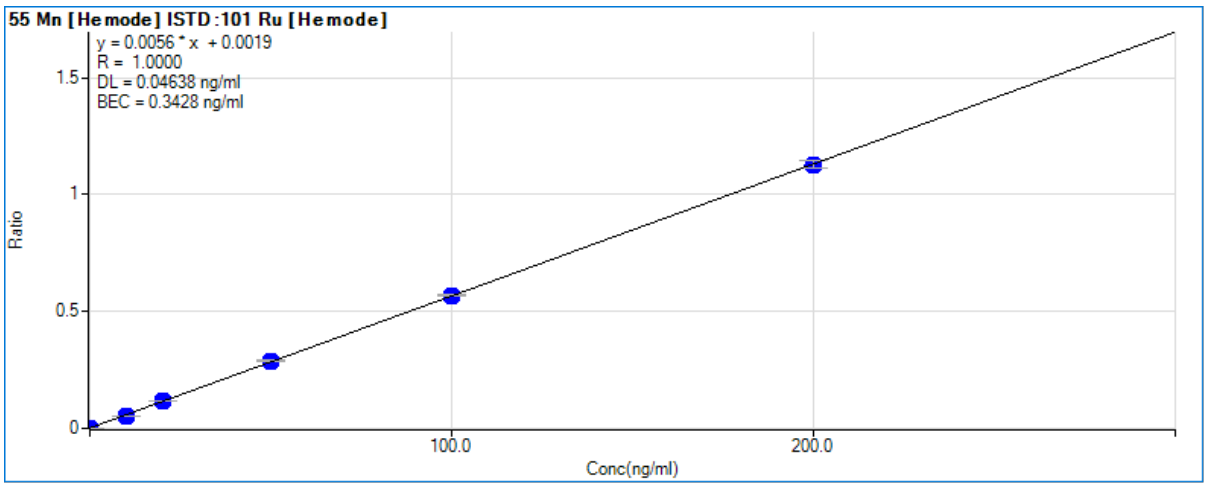
(A) Cu^{2+}



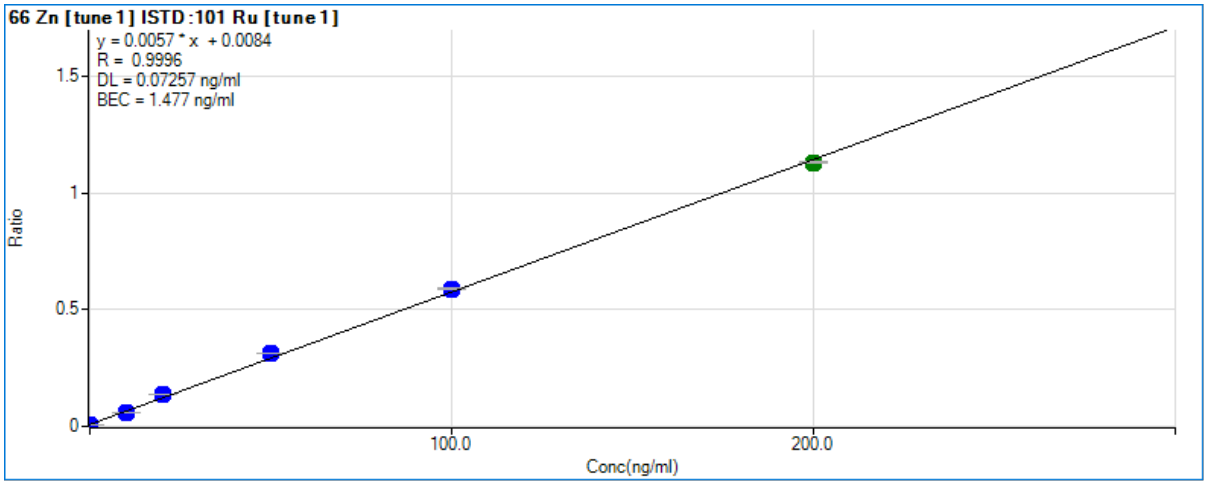
(B) Fe^{3+}



(C) Mn^{2+}



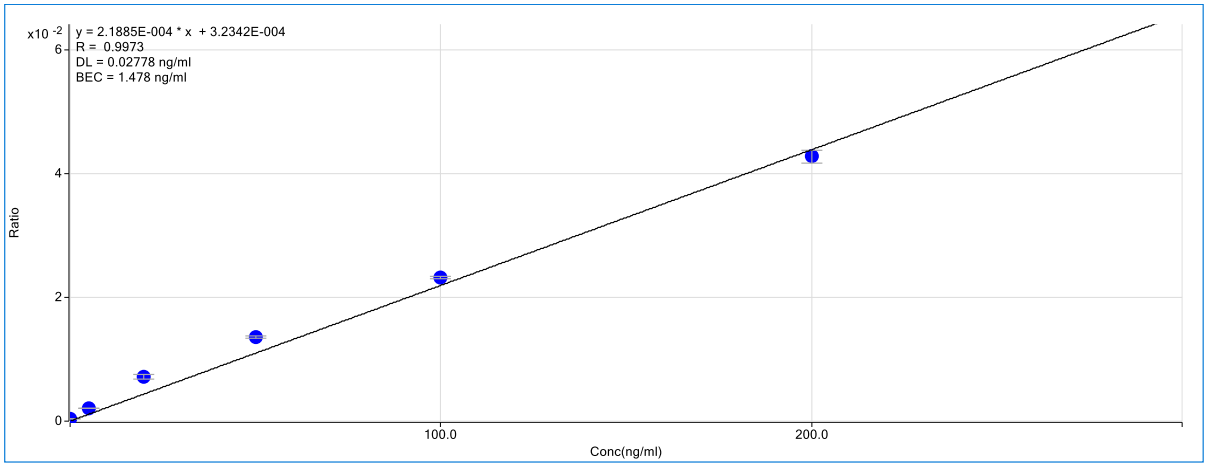
(D) Zn^{2+}


66 Zn [tune 1] ISTD :101 Ru [tune 1]
y = 0.0057 * x + 0.0084
R = 0.9996
DL = 0.07257 ng/ml
BEC = 1.477 ng/ml
Ratio
1.5
1
0.5
0
100.0
200.0
Conc(ng/ml)

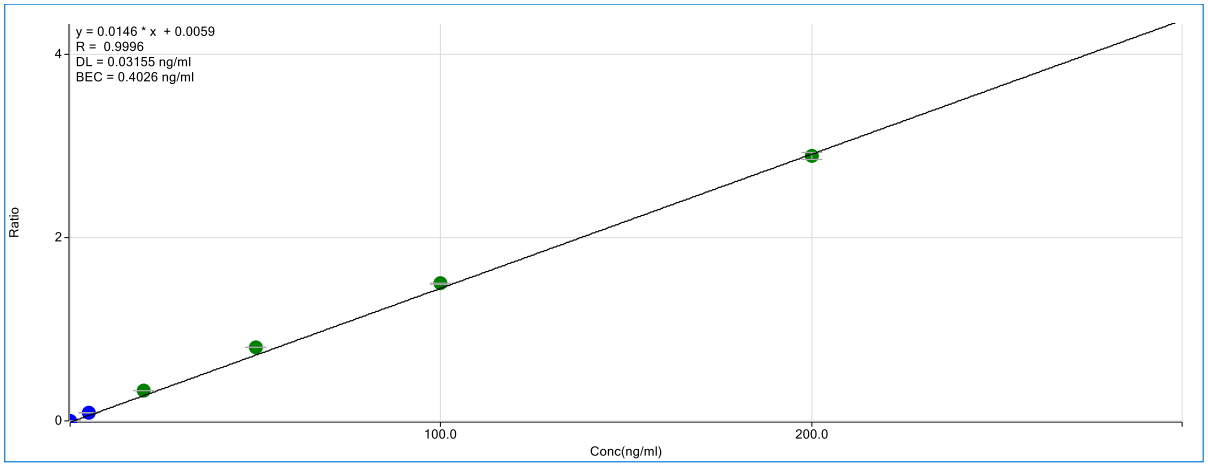
Appendix H Calibration curves for ICP-MS Analysis 2 Nasal Spray Analysis

Calibration curves for Al^{3+} (A), Cu^{2+} (B), Fe^{3+} (C) Mn^{2+} (D) and Zn^{2+} (E)
The error bars indicate the % relative standard deviation calculated during the automated calibration process using Agilent Mass Hunter software.

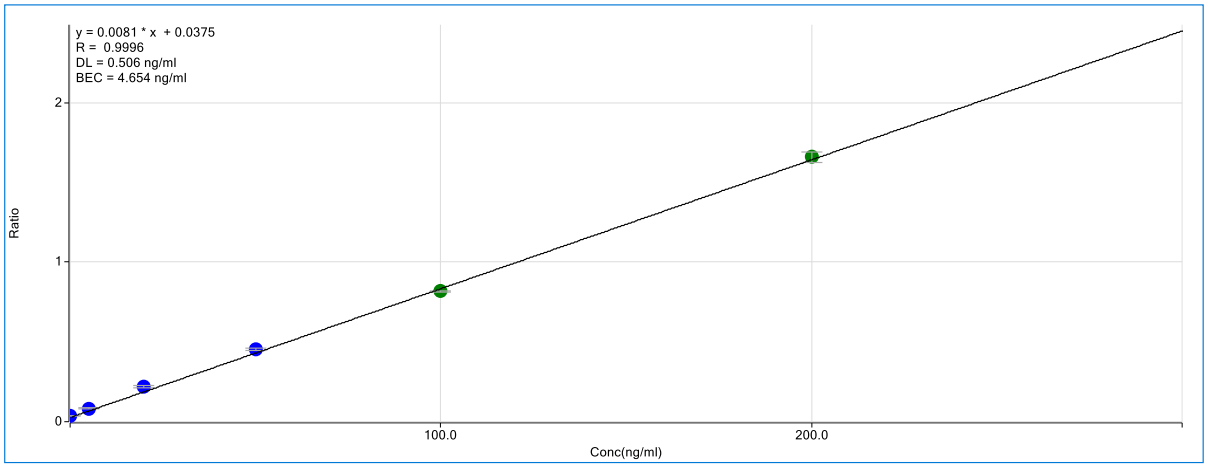
(A) Al^{3+}



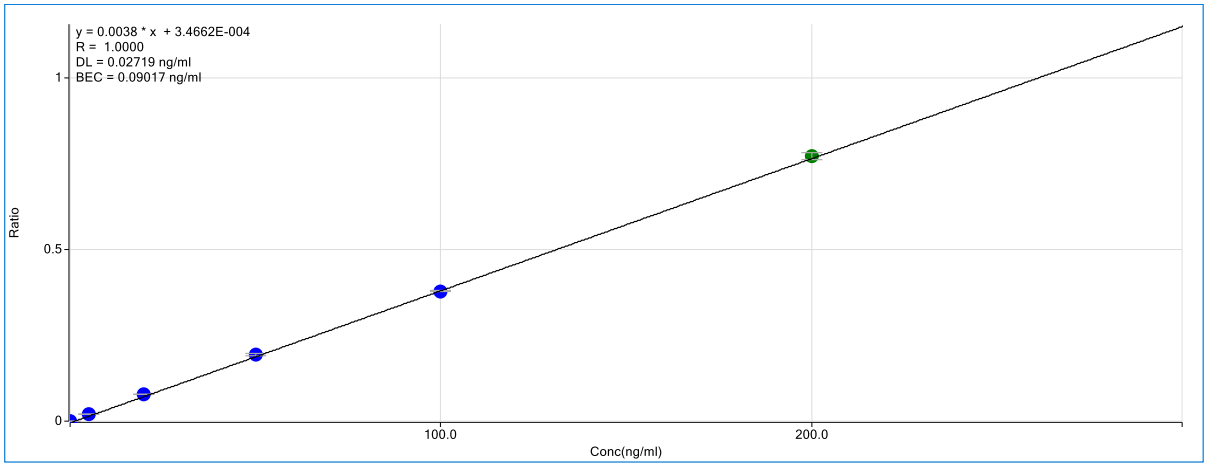
(B) Cu^{2+}



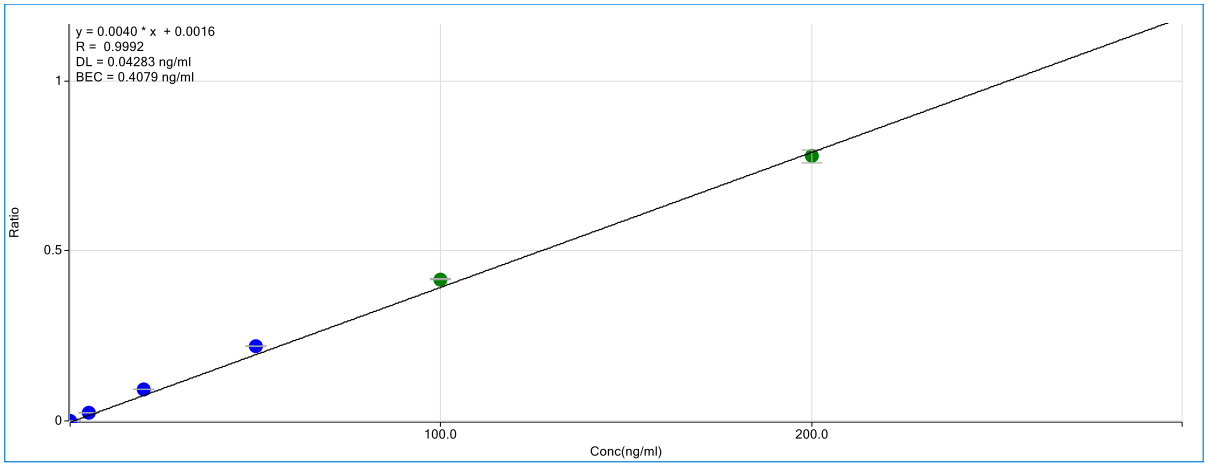
(C) Fe^{3+}



(D) Mn^{2+}


y = 0.0038 * x + 3.4662E-004
R = 1.0000
DL = 0.02719 ng/ml
BEC = 0.09017 ng/ml
Ratio
1
0.5
0
100.0
200.0
Conc(ng/ml)

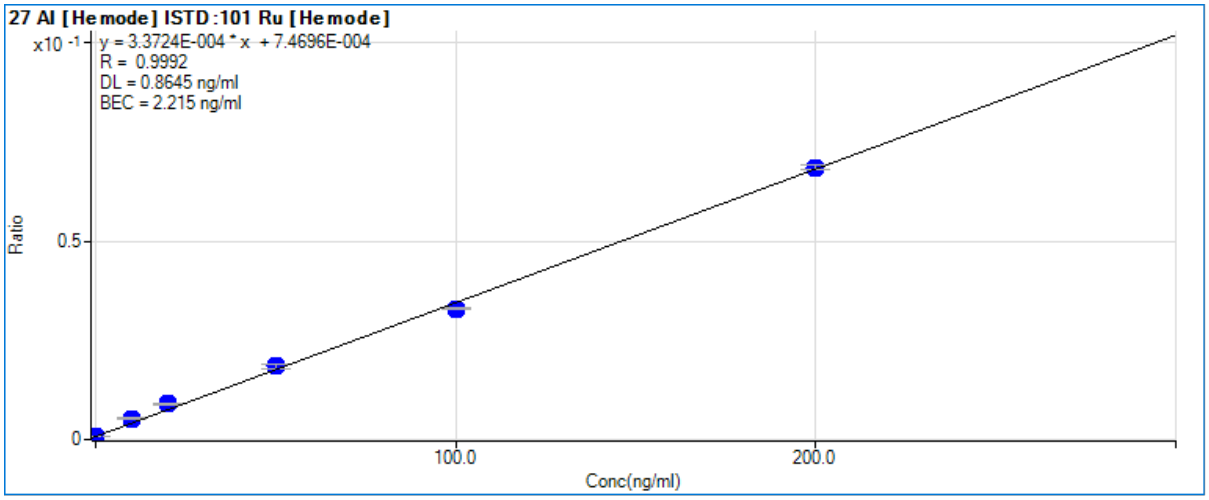
(E) Zn^{2+}


y = 0.0040 * x + 0.0016
R = 0.9992
DL = 0.04283 ng/ml
BEC = 0.4079 ng/ml
Ratio
1
0.5
0
100.0
200.0
Conc(ng/ml)

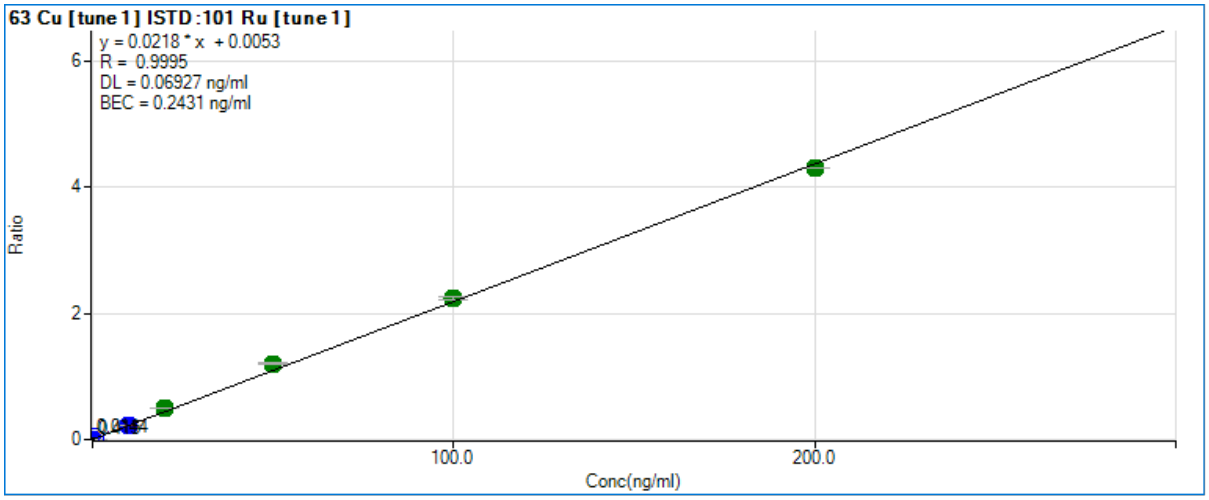
Appendix I Calibration curves for ICP-MS Analysis 1 Method development Trace metals cottonwool and nasal mucus

Calibration curves for Al^{3+} (A), Cu^{2+} (B), Fe^{3+} (C) Mn^{2+} (D) and Zn^{2+}(E)
The error bars indicate the % relative standard deviation calculated during the automated calibration process using Agilent Mass Hunter software.

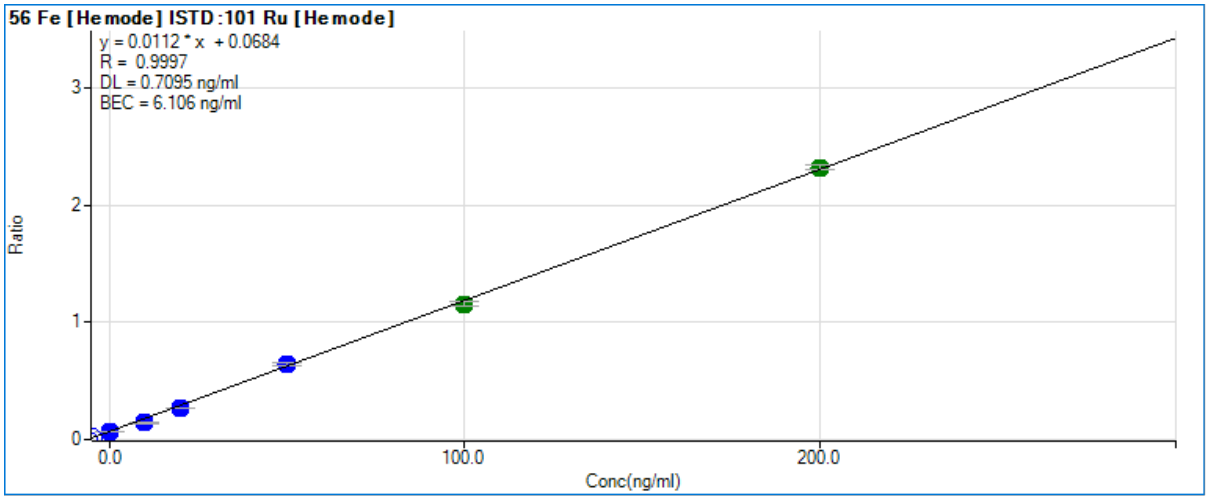
(A) Al^{3+}



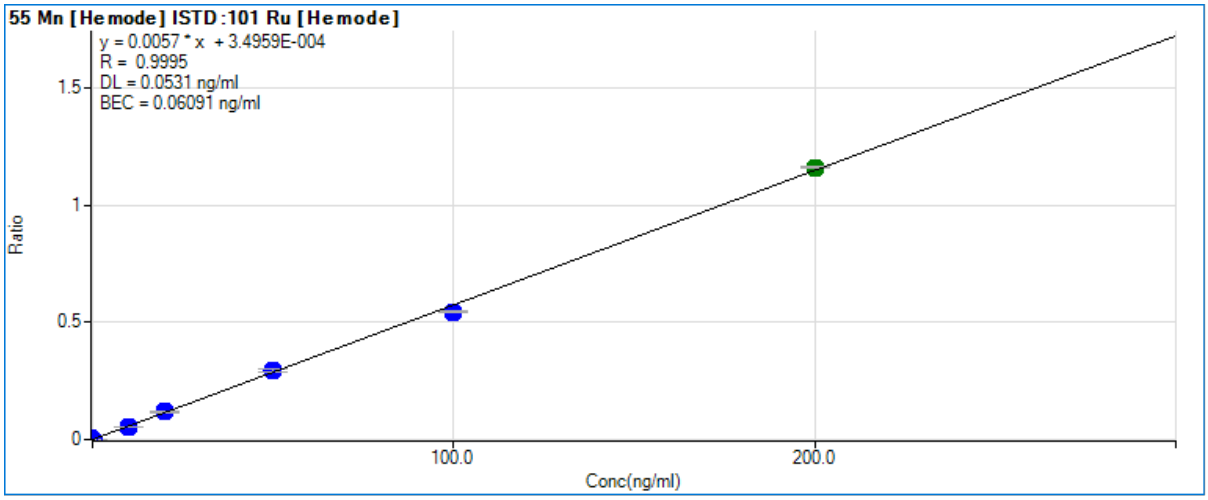
(B) Cu^{2+}



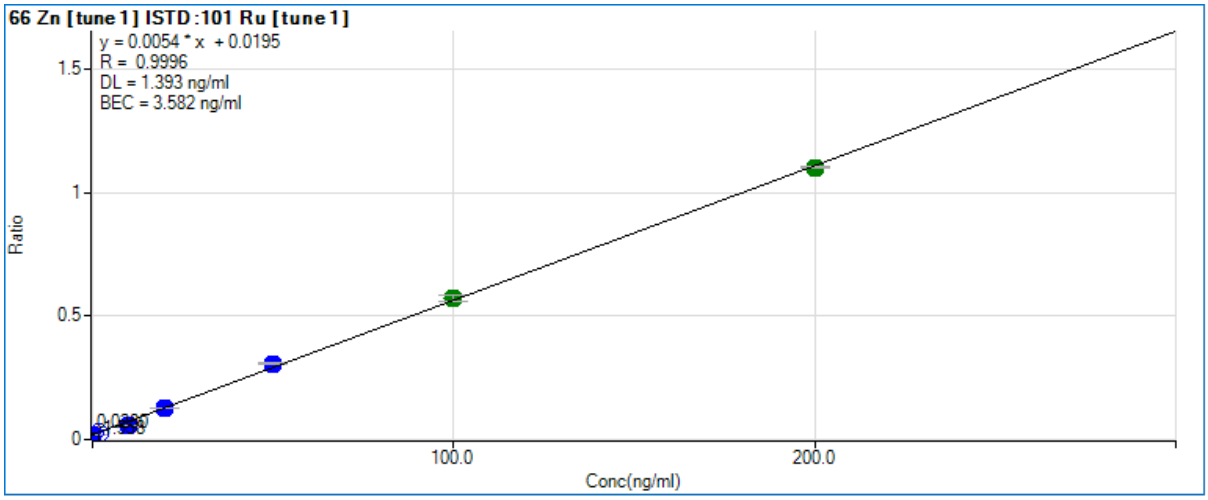
(C) Fe^{3+}



(D) Mn^{2+}


55 Mn [He mode] ISTD :101 Ru [He mode]
y = 0.0057 * x + 3.4959E-004
R = 0.9995
DL = 0.0531 ng/ml
BEC = 0.06091 ng/ml
Ratio
1.5
1
0.5
0
100.0
200.0
Conc(ng/ml)

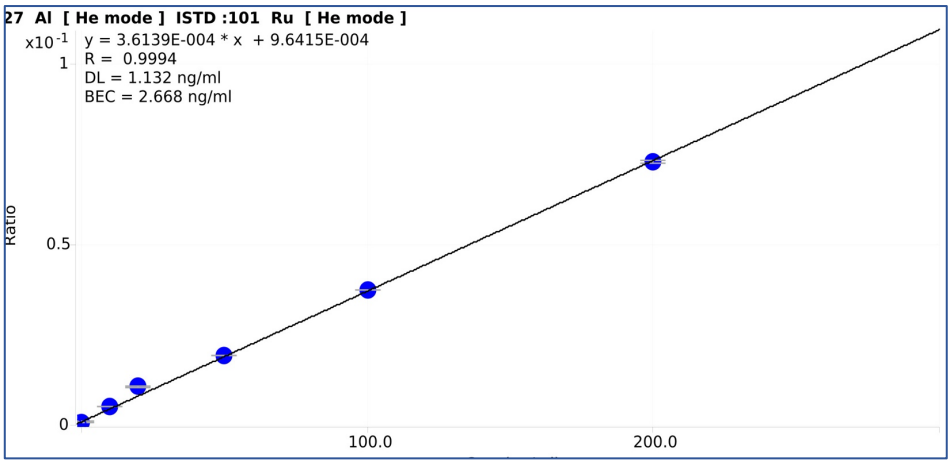
(E) Zn^{2+}


66 Zn [tune 1] ISTD :101 Ru [tune 1]
y = 0.0054 * x + 0.0195
R = 0.9996
DL = 1.393 ng/ml
BEC = 3.582 ng/ml
Ratio
1.5
1
0.5
0
100.0
200.0
Conc(ng/ml)

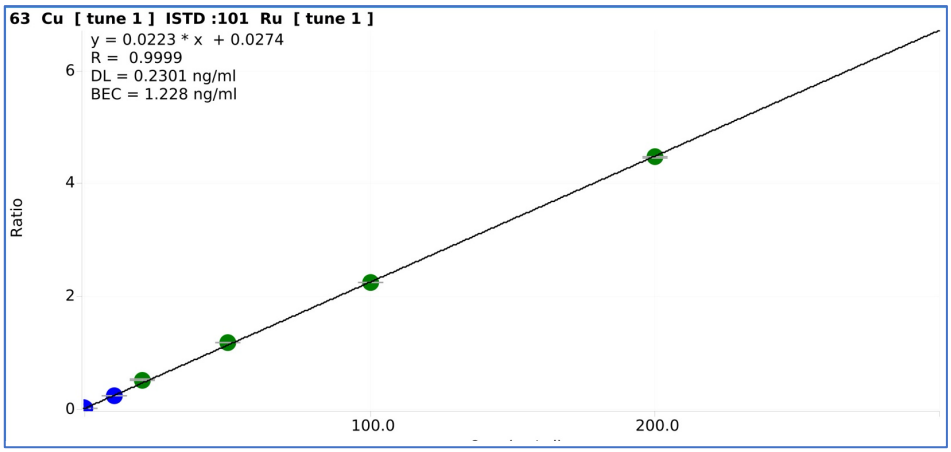
Appendix J Calibration curves for ICP-MS Analysis 2 Method development Trace metals cotton wool and nasal mucus

Calibration curves for Al^{3+} (A), Cu^{2+} (B), Fe^{3+} (C) Mn^{2+} (D) and Zn^{2+} (E)
The error bars indicate the % relative standard deviation calculated during the automated calibration process using Agilent Mass Hunter software.

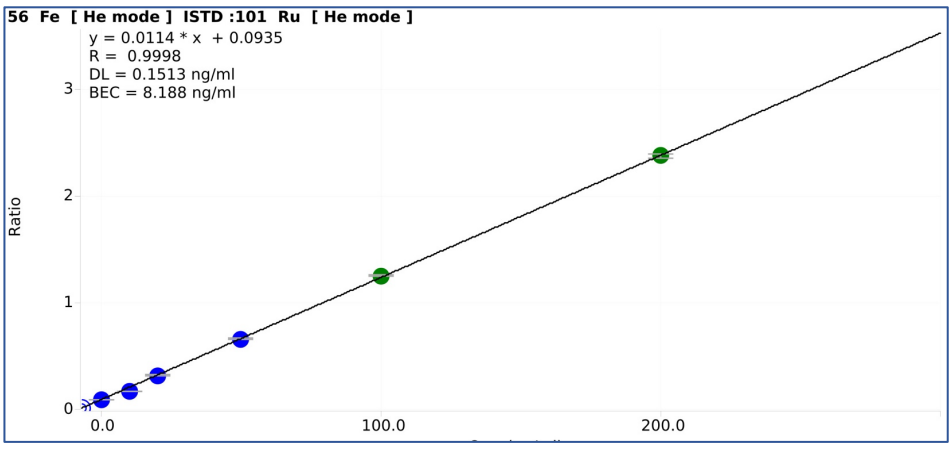
(A) Al^{3+}



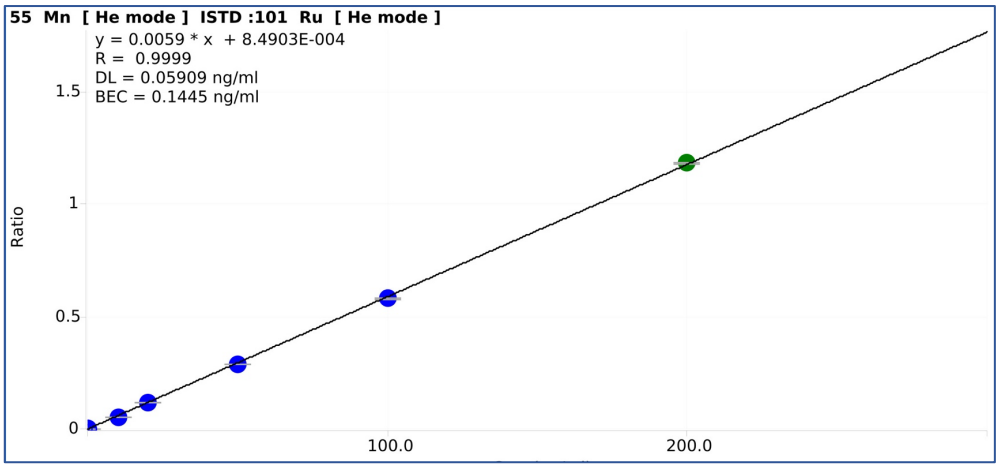
(B) Cu^{2+}



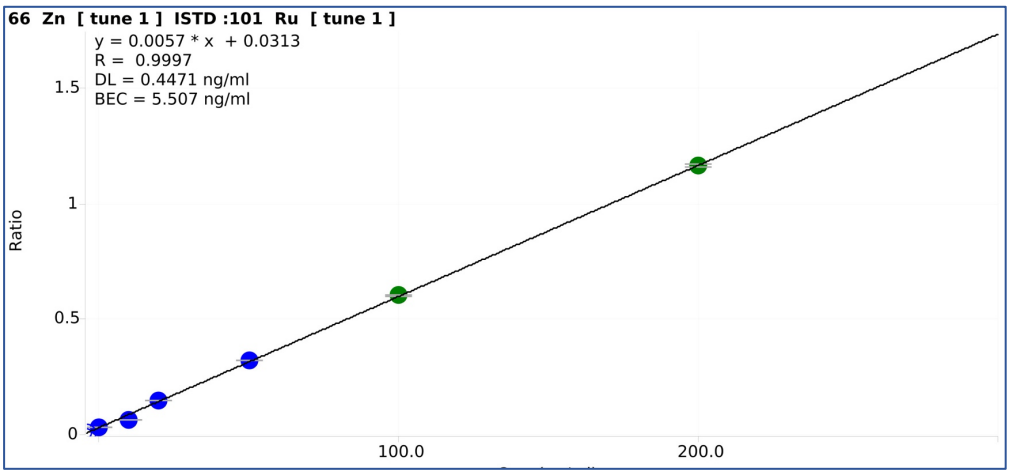
(C) Fe^{3+}



(D) Mn^{2+}

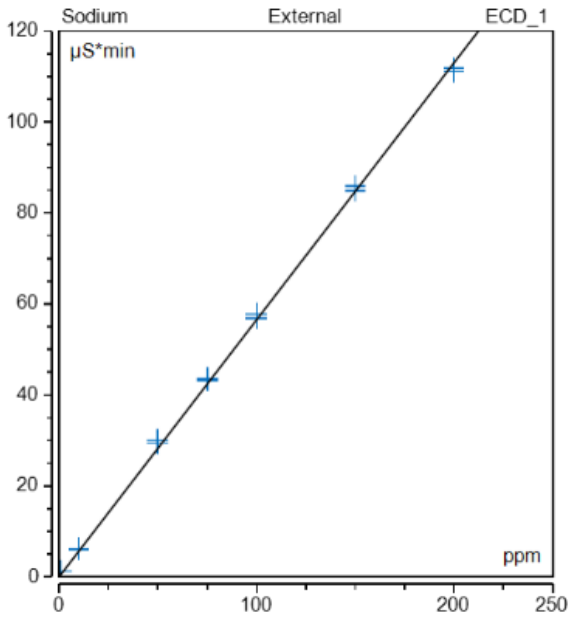

55 Mn [He mode] ISTD :101 Ru [He mode]
y = 0.0059 * x + 8.4903E-004
R = 0.9999
DL = 0.05909 ng/ml
BEC = 0.1445 ng/ml
Ratio
1.5
1
0.5
0
100.0
200.0

(E) Zn^{2+}

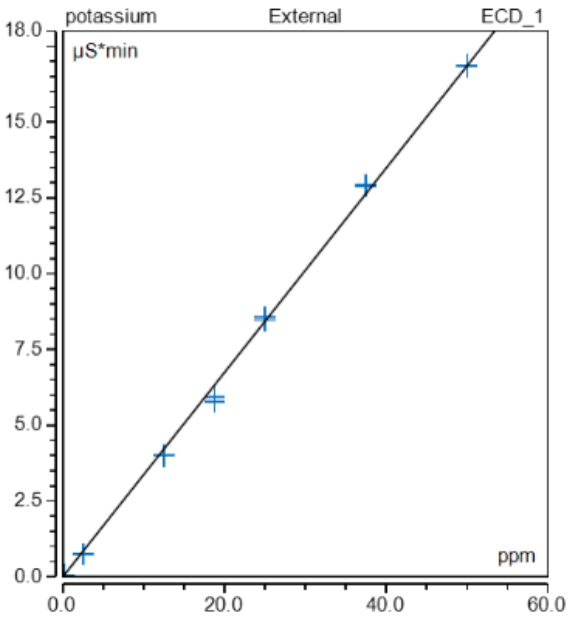

66 Zn [tune 1] ISTD :101 Ru [tune 1]
y = 0.0057 * x + 0.0313
R = 0.9997
DL = 0.4471 ng/ml
BEC = 5.507 ng/ml
Ratio
1.5
1
0.5
0
100.0
200.0

Appendix K Calibration curves IC Analysis 1 Method development bulk metals cotton wool and nasal mucus.

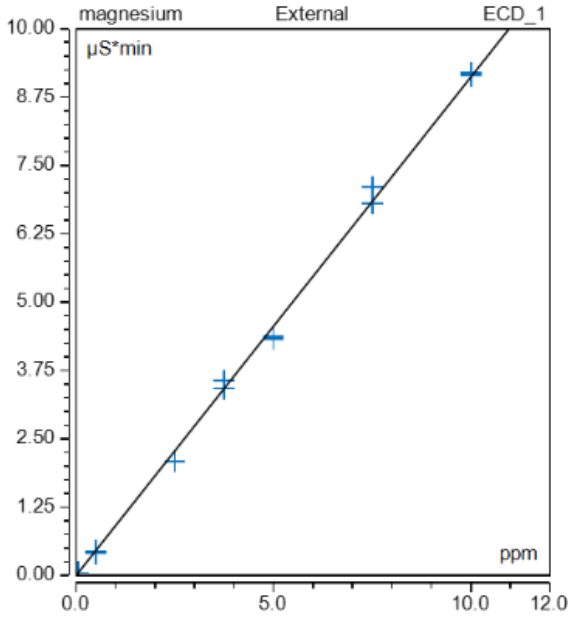
Calibration curves for (A) Na^+, (B) K^+, (C) Mg^{2+} and (D) Ca^{2+}


Sodium
External
ECD_1
µS*min
ppm
120
100
80
60
40
20
0
0
100
200
250

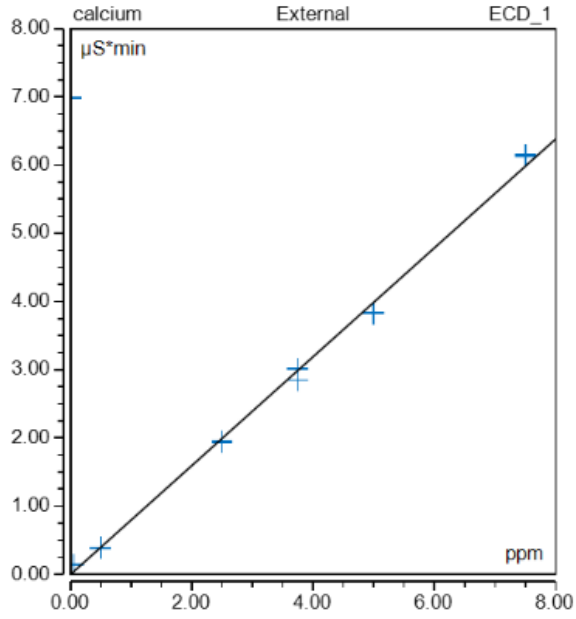
A


potassium
External
ECD_1
µS*min
ppm
18.0
15.0
12.5
10.0
7.5
5.0
2.5
0.0
0.0
20.0
40.0
60.0

B


magnesium
External
ECD_1
µS*min
ppm
10.00
8.75
7.50
6.25
5.00
3.75
2.50
1.25
0.00
0.0
5.0
10.0
12.0

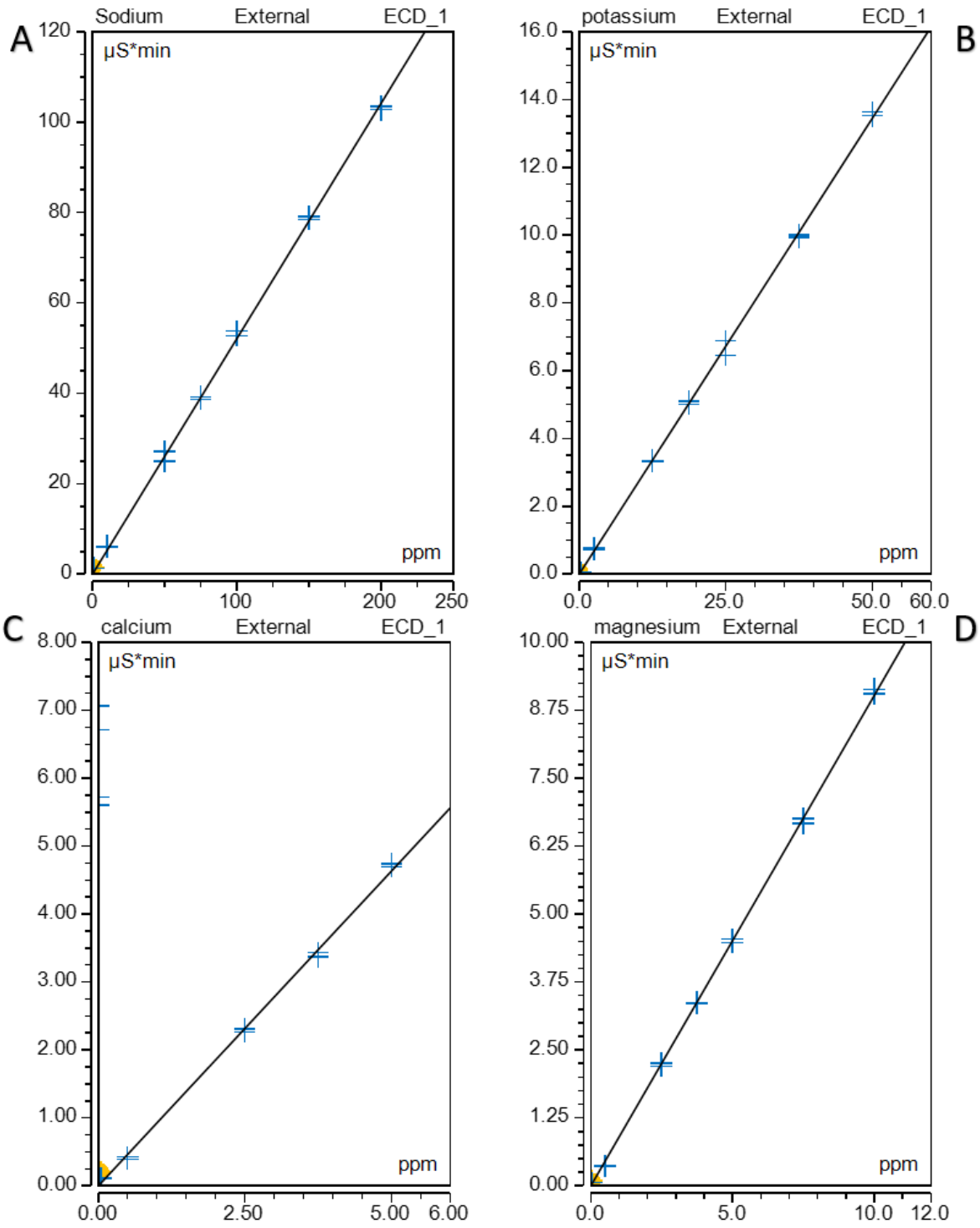
C


calcium
External
ECD_1
µS*min
ppm
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0.00
2.00
4.00
6.00
8.00

D

Appendix L Calibration curves IC analysis 2 method development bulk metals cottonwool and nasal mucus

Na^+ (A), K^+ (B), Ca^{2+} (C) Mg^{2+} (D)

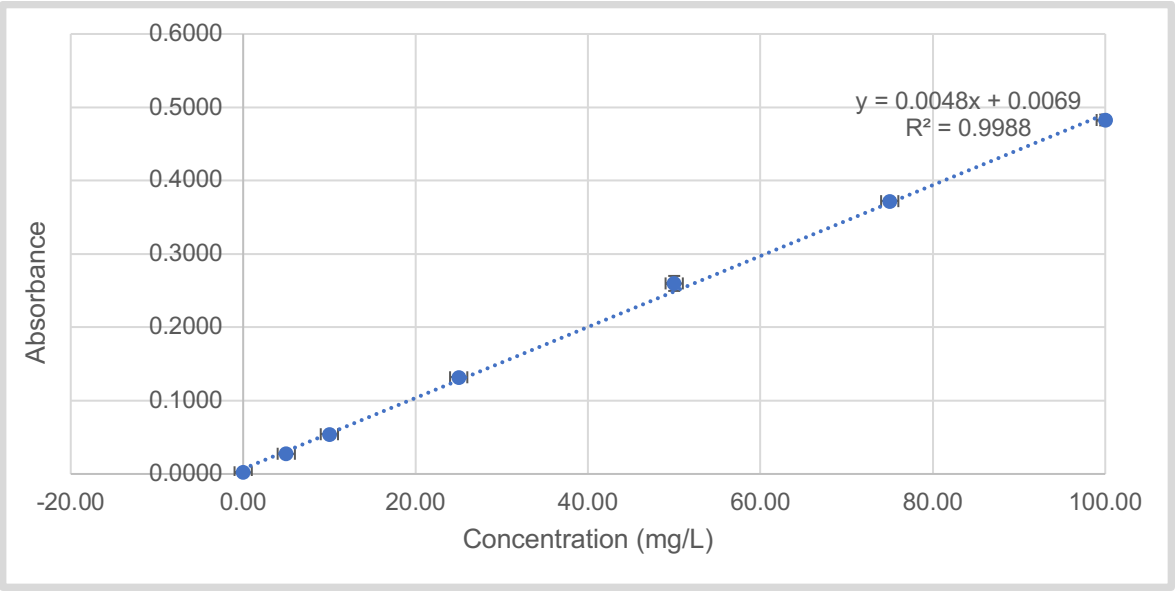

A
Sodium External ECD_1
µS*min
ppm
120
100
80
60
40
20
0
0
100
200
250
B
potassium External ECD_1
µS*min
ppm
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0.0
25.0
50.0
60.0
C
calcium External ECD_1
µS*min
ppm
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0.00
2.50
5.00
6.00
D
magnesium External ECD_1
µS*min
ppm
10.00
8.75
7.50
6.25
5.00
3.75
2.50
1.25
0.00
0.0
5.0
10.0
12.0

Appendix M Calibration curves AAS 1 method development cottonwool and nasal mucus

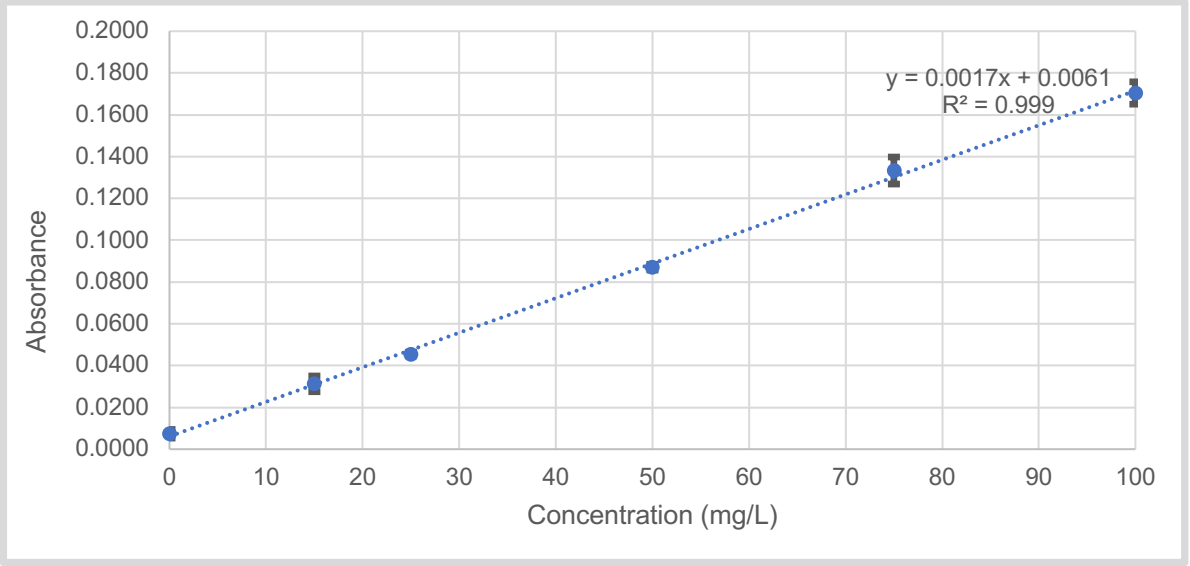
Calibration curves for Na^+ (A), K^+ (B), Ca^{2+} (C), Mg^{2+} (D)

The error bars indicate the standard deviation of 3 replicates during the calibration process.

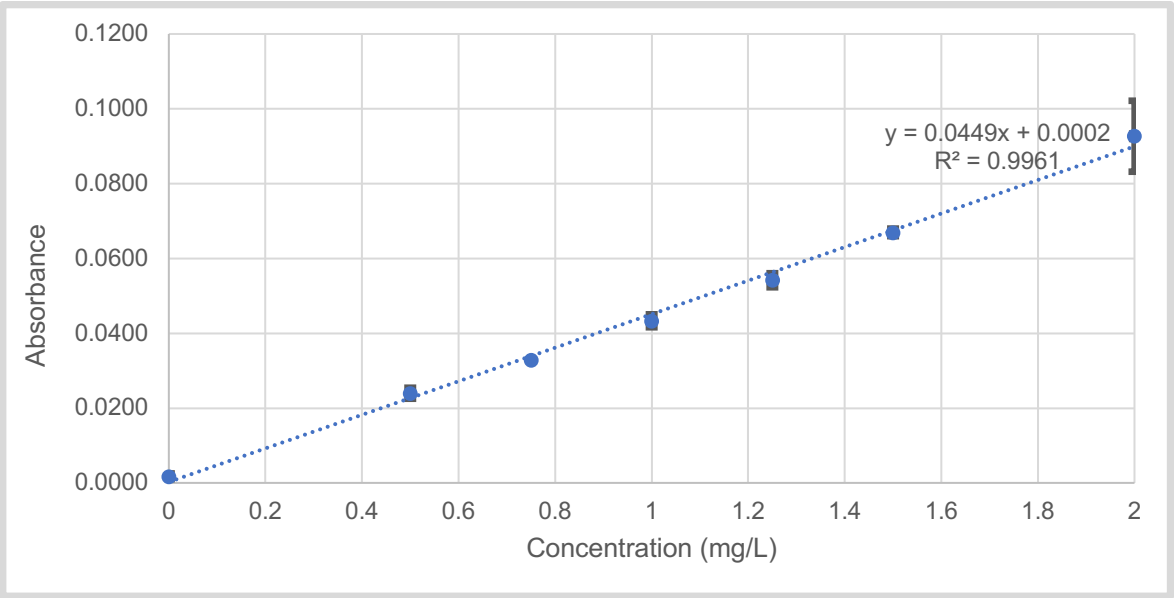
(A) Na^+


y = 0.0048x + 0.0069
R² = 0.9988
Absorbance
Concentration (mg/L)
0.6000
0.5000
0.4000
0.3000
0.2000
0.1000
0.0000
-20.00
0.00
20.00
40.00
60.00
80.00
100.00

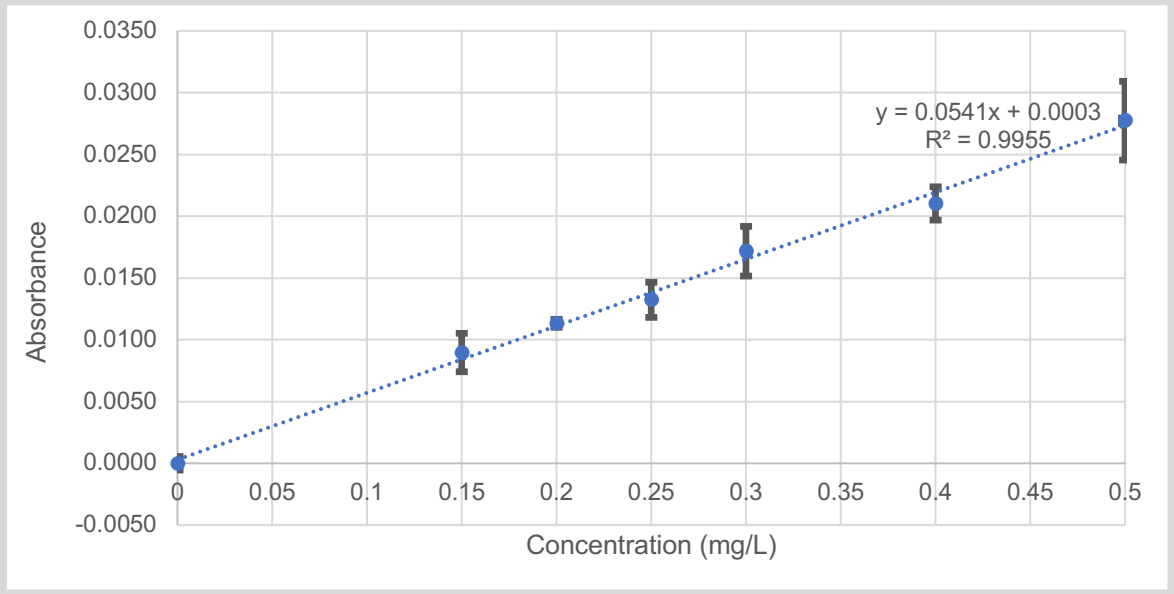
(B) K^+


y = 0.0017x + 0.0061
R² = 0.999
Absorbance
Concentration (mg/L)
0.2000
0.1800
0.1600
0.1400
0.1200
0.1000
0.0800
0.0600
0.0400
0.0200
0.0000
0
10
20
30
40
50
60
70
80
90
100

(C) Ca^{2+}


0.1200
0.1000
0.0800
0.0600
0.0400
0.0200
0.0000
Absorbance
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
Concentration (mg/L)
y = 0.0449x + 0.0002
R² = 0.9961

(D) Mg^{2+}

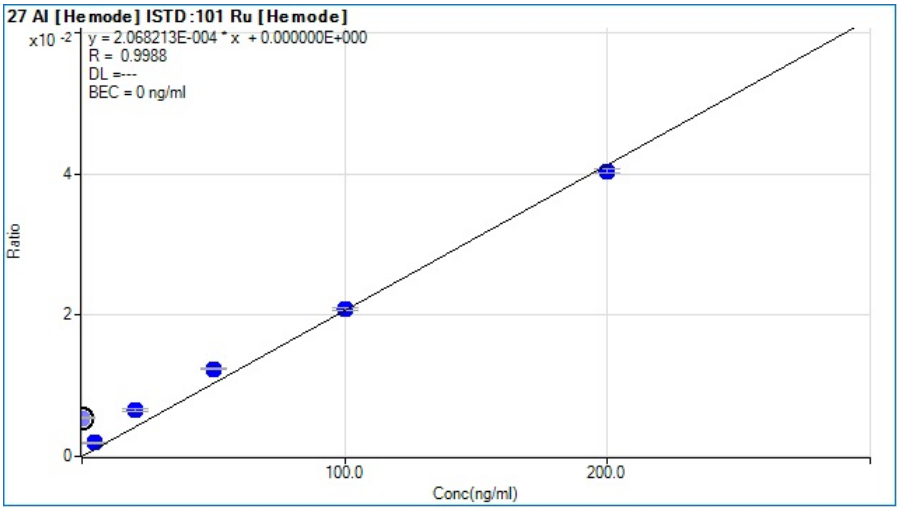

0.0350
0.0300
0.0250
0.0200
0.0150
0.0100
0.0050
0.0000
-0.0050
Absorbance
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
Concentration (mg/L)
y = 0.0541x + 0.0003
R² = 0.9955

Appendix N Calibration Curves ICP-MS Cotton Wool and Nasal Mucus Analysis 80 Samples

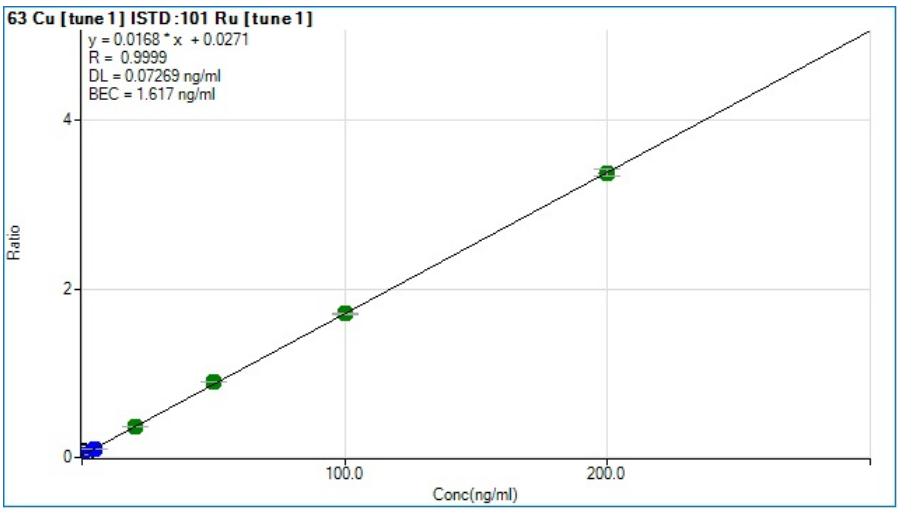
Calibration curves for Al^{3+}, Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+}

The error bars indicate the standard deviation of 3 replicates during the calibration process.

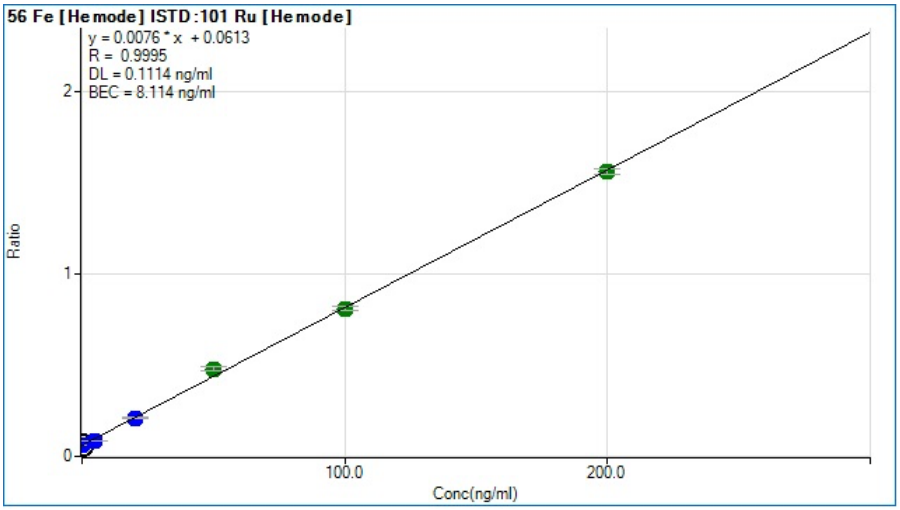
(A) Al^{3+}



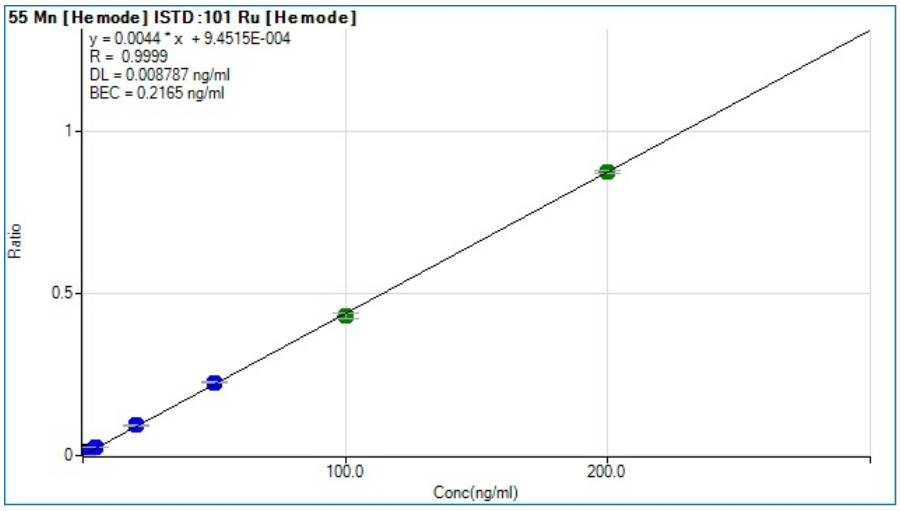
(B) Cu^{2+}



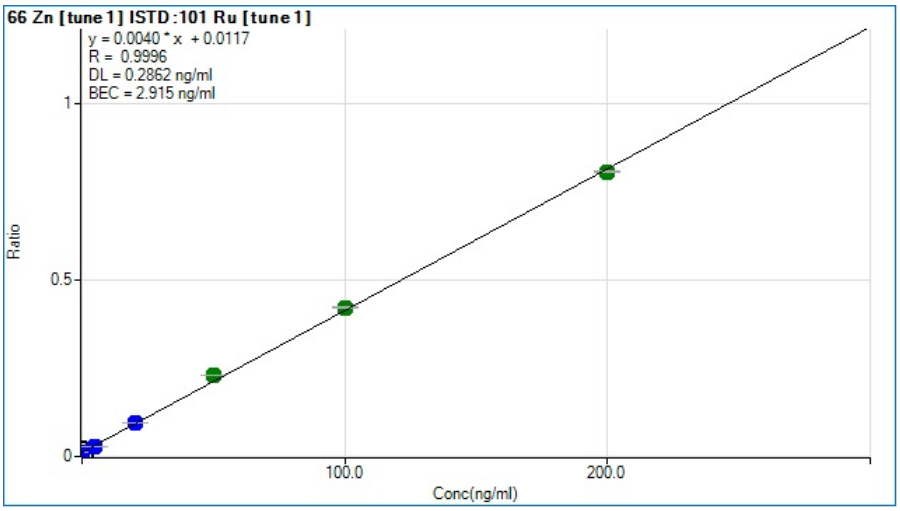
(C) Fe^{3+}



(D) Mn^{2+}


55 Mn [Hemode] ISTD:101 Ru [Hemode]
y = 0.0044 * x + 9.4515E-004
R = 0.9999
DL = 0.008787 ng/ml
BEC = 0.2165 ng/ml
Ratio
1
0.5
0
100.0
200.0
Conc(ng/ml)

(E) Zn^{2+}

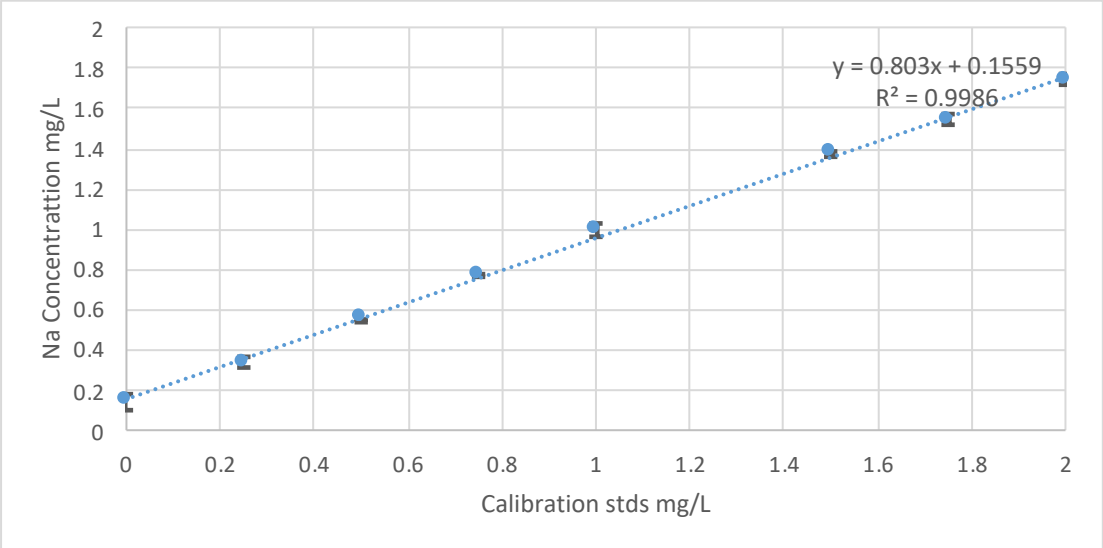

66 Zn [tune 1] ISTD:101 Ru [tune 1]
y = 0.0040 * x + 0.0117
R = 0.9996
DL = 0.2862 ng/ml
BEC = 2.915 ng/ml
Ratio
1
0.5
0
100.0
200.0
Conc(ng/ml)

Appendix O Calibration Curves AAS Nasal Mucus Analysis 80 Samples

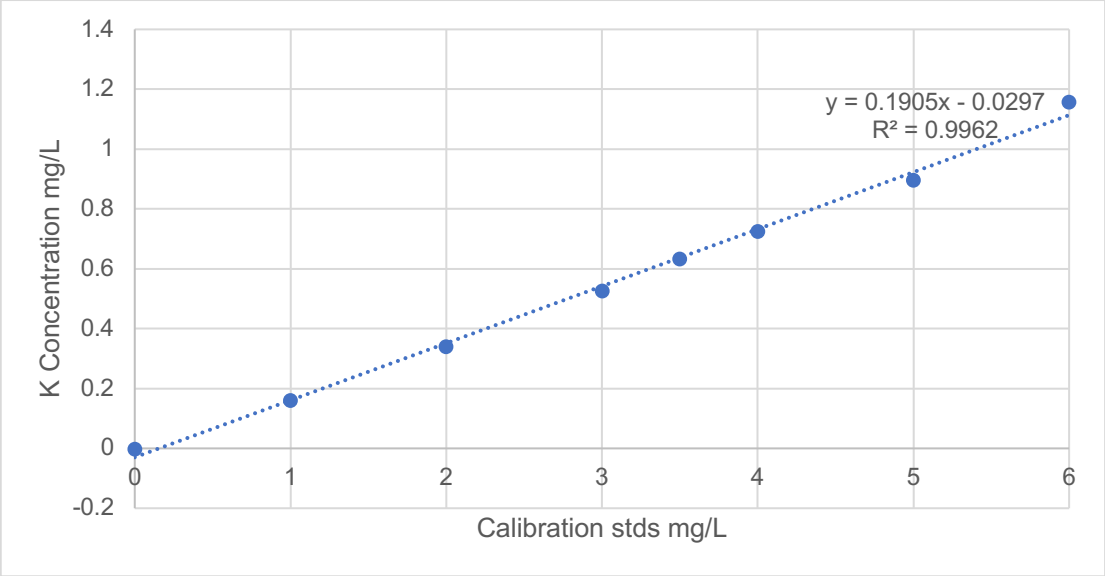
Calibration curves for (A) Na^+, (B) K^+, (C) Mg^{2+} and (D) Ca^{2+}

The error bars indicate the standard deviation of 3 replicates during the calibration process.

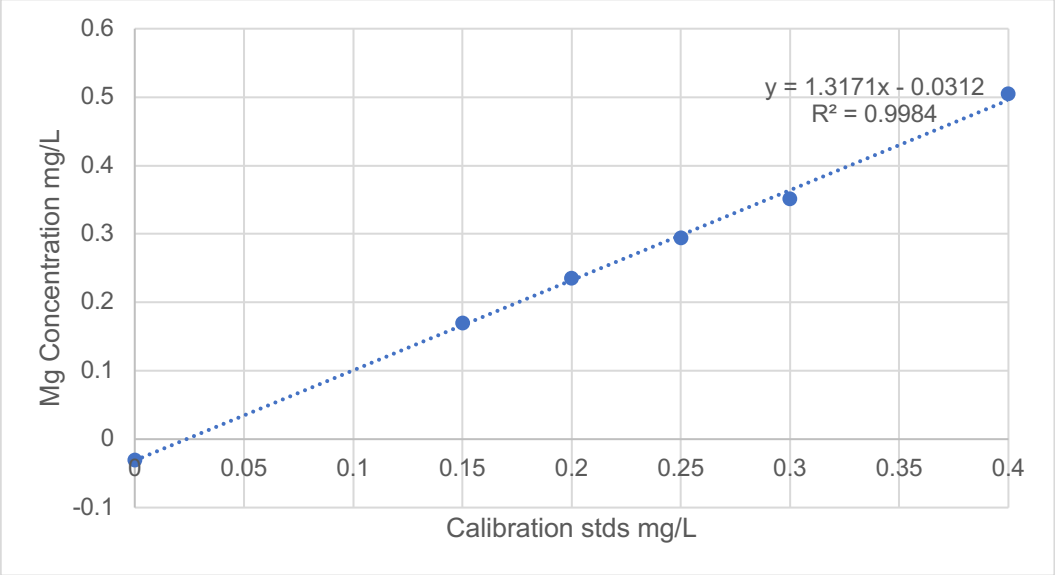
(A) Na^+


y = 0.803x + 0.1559
R² = 0.9986
Na Concentrattion mg/L
Calibration stds mg/L
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2

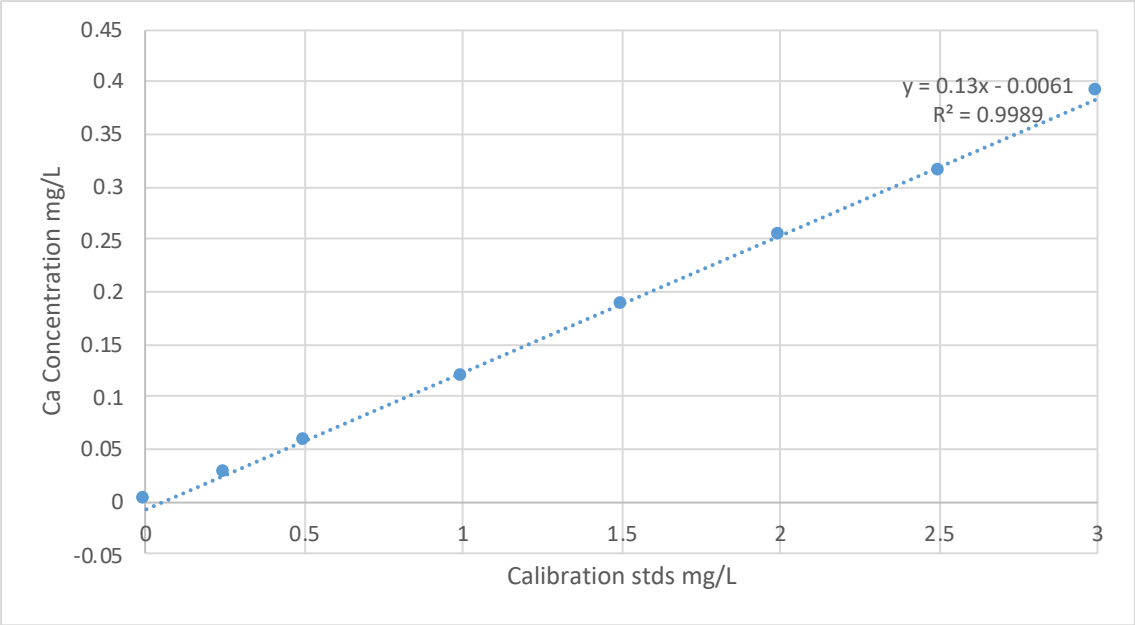
(B) K^+


y = 0.1905x - 0.0297
R² = 0.9962
K Concentration mg/L
Calibration stds mg/L
-0.2
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
1
2
3
4
5
6

(C) Mg^{2+}


y = 1.3171x - 0.0312
R² = 0.9984
Mg Concentration mg/L
Calibration stds mg/L
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4

(D) Ca^{2+}


y = 0.13x - 0.0061
R² = 0.9989
Ca Concentration mg/L
Calibration stds mg/L
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
0
0.5
1
1.5
2
2.5
3

Appendix P Full ICP-MS Results Tables for All Trace Metals in the 80 Mucus Samples

Results tables for (a) $Al^{3+,}$ (b) Cu^{2+}, (c) Fe^{3+} (d) Mn^{2+} and (e) Zn^{2+}

Negative values can be seen in a small number of samples once the average calculated mass of the cotton wool pellets were removed. It was not possible to calculate an exact mass for the cations in each individual pellet.

(a) Al^{3+}

	Cotton Wool Mass (g)	Al^{3+} Conc CW (ng/mL)	SD	Mucus Mass (g)	Al^{3+}Conc ng/mL	%RSD	SD	Sample-CW ng/mL	SD	Sample-CW umol/ml	SD umol/ml	Al^{3+} Sample-CW ng/mL X10	Al^{3+} SD ng/mL X10	Al^{3+} Sample-CW umol/L X10	Al^{3+} SD umol/ml X10	Al^{3+} Sample-CW ng/mL per g mucus	Al^{3+} SD ng/mL per g mucus	Al^{3+} Sample-CW umol/L per g mucus	Al^{3+} SD umol/L per g mucus	Sex
Sample 120015	0.15	356.93	47.79	0.38	339.98	1.62	5.49	-16.96	53.28	-0.63	1.97	-169.55	532.78	-6.28	19.75	-446.18	1402.06	-16.54	51.97	F
Sample 120016	0.10	237.96	31.86	0.38	326.97	0.77	2.53	89.01	34.39	3.30	1.27	890.09	343.90	32.99	12.75	2342.34	905.01	86.82	33.54	
Sample 120017	0.13	309.34	41.41	0.74	473.47	0.82	3.90	164.12	45.31	6.08	1.68	1641.25	453.14	60.83	16.80	2217.90	612.35	82.21	22.70	F
Sample 120018	0.10	237.96	31.86	0.75	713.52	0.32	2.26	475.56	34.12	17.63	1.26	4755.63	341.22	176.27	12.65	6340.84	454.96	235.02	16.86	
Sample 120019	0.12	285.55	38.23	0.39	512.61	1.22	6.24	227.07	44.46	8.42	1.65	2270.66	444.64	84.16	16.48	5822.20	1140.10	215.80	42.26	M
Sample 120020	0.22	523.50	70.08	0.33	549.57	0.09	0.47	26.07	70.56	0.97	2.62	260.71	705.58	9.66	26.15	790.02	2138.14	29.28	79.25	
Sample 120021	0.13	309.34	41.41	0.39	549.00	1.18	6.49	239.66	47.91	8.88	1.78	2396.55	479.08	88.83	17.76	6145.01	1228.41	227.76	45.53	M
Sample 120022	0.13	309.34	41.41	0.46	484.12	1.21	5.85	174.78	47.26	6.48	1.75	1747.81	472.61	64.78	17.52	3799.58	1027.42	140.83	38.08	
Sample 120023	0.11	261.75	35.04	0.33	417.92	1.78	7.45	156.17	42.49	5.79	1.57	1561.66	424.88	57.88	15.75	4732.30	1287.52	175.40	47.72	F
Sample 120024	0.14	333.14	44.60	0.33	554.88	0.07	0.39	221.74	44.99	8.22	1.67	2217.43	449.86	82.19	16.67	6719.50	1363.20	249.05	50.53	
Sample 120025	0.11	261.75	35.04	0.23	349.37	1.83	6.38	87.61	41.43	3.25	1.54	876.14	414.25	32.47	15.35	3809.30	1801.11	141.19	66.76	M
Sample 120026	0.11	261.75	35.04	0.31	437.65	0.90	3.93	175.90	38.97	6.52	1.44	1759.01	389.70	65.20	14.44	5674.21	1257.09	210.31	46.59	
Sample 120027	0.12	285.55	38.23	0.62	2459.89	0.79	19.43	2174.34	57.66	80.59	2.14	21743.43	576.62	805.91	21.37	35070.05	930.03	1299.85	34.47	M
Sample 120028	0.13	309.34	41.41	0.51	1797.26	0.11	1.96	1487.92	43.37	55.15	1.61	14879.22	433.73	551.49	16.08	29174.94	850.45	1081.35	31.52	
Sample 120029	0.12	285.55	38.23	0.39	1366.64	0.19	2.56	1081.09	40.79	40.07	1.51	10810.88	407.92	400.70	15.12	27720.20	1045.95	1027.44	38.77	F
Sample 120030	0.12	285.55	38.23	0.32	545.15	1.89	10.28	259.60	48.51	9.62	1.80	2595.98	485.07	96.22	17.98	8112.43	1515.86	300.68	56.18	
Sample 120031	0.12	285.55	38.23	0.18	527.59	1.11	5.88	242.04	44.11	8.97	1.63	2420.42	441.10	89.71	16.35	13446.77	2450.57	498.40	90.83	M
Sample 120032	0.13	309.34	41.41	0.20	469.35	0.39	1.84	160.00	43.25	5.93	1.60	1600.04	432.55	59.30	16.03	8000.19	2162.73	296.52	80.16	
Sample 120033	0.14	333.14	44.60	0.27	487.92	1.03	5.01	154.78	49.61	5.74	1.84	1547.80	496.08	57.37	18.39	5732.60	1837.34	212.48	68.10	F
Sample 120034	0.12	285.55	38.23	0.22	523.72	0.19	0.98	238.17	39.21	8.83	1.45	2381.71	392.10	88.28	14.53	10825.98	1782.28	401.26	66.06	
Sample 120035	0.13	309.34	41.41	0.50	1466.69	2.27	33.32	1157.34	74.74	42.90	2.77	11573.44	747.35	428.96	27.70	23146.88	1494.71	857.93	55.40	F
Sample 120036	0.12	285.55	38.23	0.37	469.13	0.37	1.74	183.58	39.96	6.80	1.48	1835.81	399.65	68.04	14.81	4961.64	1080.13	183.90	40.03	
Sample 120037	0.13	309.34	41.41	0.29	412.46	0.70	2.90	103.11	44.32	3.82	1.64	1031.12	443.16	38.22	16.43	3555.60	1528.15	131.79	56.64	F
Sample 120038	0.12	285.55	38.23	0.21	451.12	0.34	1.53	165.58	39.76	6.14	1.47	1655.77	397.59	61.37	14.74	7884.61	1893.27	292.24	70.17	
Sample 120039	0.12	285.55	38.23	0.20	425.95	1.24	5.29	140.41	43.52	5.20	1.61	1404.06	435.16	52.04	16.13	7020.31	2175.80	260.20	80.65	M
Sample 120040	0.13	309.34	41.41	0.17	867.52	2.64	22.88	558.18	64.30	20.69	2.38	5581.80	642.96	206.89	23.83	32834.15	3782.14	1216.98	140.18	
Sample 120041	0.12	285.55	38.23	0.32	657.48	1.69	11.12	371.93	49.35	13.79	1.83	3719.33	493.47	137.85	18.29	11622.89	1542.09	430.80	57.16	F

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	Cotton Wool Mass (g)	Al^{3+} Conc CW (ng/mL)	SD	Mucus Mass (g)	Al^{3+}Conc ng/mL	%RSD	SD	Sample-CW ng/mL	SD	Sample-CW umol/ml	SD umol/ml	Al^{3+} Sample-CW ng/mL X10	Al^{3+} SD ng/mL X10	Al^{3+} Sample-CW umol/L X10	Al^{3+} SD umol/ml X10	Al^{3+} Sample-CW ng/mL per g mucus	Al^{3+} SD ng/mL per g mucus	Al^{3+} Sample-CW umol/L per g mucus	Al^{3+} SD umol/L per g mucus	Sex
Sample 120042	0.13	309.34	41.41	0.21	590.13	1.35	7.96	280.79	49.37	10.41	1.83	2807.88	493.72	104.07	18.30	13370.84	2351.04	495.58	87.14	
Sample 120043	0.15	356.93	47.79	0.20	500.03	0.57	2.84	143.10	50.62	5.30	1.88	1430.97	506.23	53.04	18.76	7154.87	2531.13	265.19	93.82	F
Sample 120044	0.14	333.14	44.60	0.18	472.00	1.93	9.11	138.86	53.71	5.15	1.99	1388.56	537.14	51.47	19.91	7714.24	2984.11	285.92	110.60	
Sample 120045	0.12	285.55	38.23	0.16	543.97	3.33	18.11	258.42	56.34	9.58	2.09	2584.22	563.38	95.78	20.88	16151.36	3521.11	598.64	130.51	F
Sample 120046	0.14	333.14	44.60	0.10	906.13	0.82	7.43	572.99	52.02	21.24	1.93	5729.91	520.25	212.38	19.28	57299.05	5202.45	2123.76	192.83	
Sample 120047	0.12	285.55	38.23	0.20	370.67	1.50	5.55	85.12	43.78	3.16	1.62	851.25	437.81	31.55	16.23	4256.24	2189.06	157.76	81.14	M
Sample 120048	0.13	309.34	41.41	0.12	341.25	0.95	3.24	31.91	44.65	1.18	1.65	319.10	446.51	11.83	16.55	2659.19	3720.92	98.56	137.91	
Sample 120049	0.11	261.75	35.04	0.22	379.53	1.20	4.55	117.78	39.59	4.37	1.47	1177.83	395.93	43.66	14.67	5353.76	1799.67	198.43	66.70	F
Sample 120050	0.13	309.34	41.41	0.40	356.50	1.36	4.86	47.16	46.27	1.75	1.72	471.60	462.75	17.48	17.15	1179.00	1156.87	43.70	42.88	
Sample 120051	0.11	261.75	35.04	0.15	612.03	1.73	10.59	350.28	45.63	12.98	1.69	3502.79	456.32	129.83	16.91	23351.91	3042.12	865.53	112.75	M
Sample 120052	0.12	285.55	38.23	0.15	576.36	2.05	11.80	290.81	50.02	10.78	1.85	2908.08	500.25	107.79	18.54	19387.17	3334.98	718.58	123.61	
Sample 120053	0.14	333.14	44.60	0.14	454.59	0.78	3.56	121.45	48.16	4.50	1.78	1214.55	481.56	45.02	17.85	8675.33	3439.74	321.55	127.49	M
Sample 120054	0.13	309.34	41.41	0.36	372.03	2.25	8.37	62.69	49.78	2.32	1.85	626.90	497.82	23.24	18.45	1741.38	1382.84	64.54	51.25	
Sample 120055	0.12	285.55	38.23	0.40	549.55	0.04	0.24	264.00	38.47	9.79	1.43	2640.04	384.69	97.85	14.26	6600.11	961.72	244.63	35.65	F
Sample 120056	0.13	309.34	41.41	0.34	349.84	0.29	1.01	40.50	42.42	1.50	1.57	405.00	424.19	15.01	15.72	1191.19	1247.62	44.15	46.24	
Sample 120057	0.12	285.55	38.23	0.37	355.55	0.03	0.12	70.00	38.35	2.59	1.42	699.98	383.52	25.94	14.22	1891.84	1036.55	70.12	38.42	M
Sample 120058	0.13	309.34	41.41	0.52	366.84	0.71	2.62	57.50	44.03	2.13	1.63	574.99	440.30	21.31	16.32	1105.75	846.73	40.98	31.38	
Sample 120059	0.13	309.34	41.41	0.43	310.60	1.43	4.43	1.26	45.84	0.05	1.70	12.55	458.41	0.47	16.99	29.19	1066.07	1.08	39.51	M
Sample 120060	0.13	309.34	41.41	0.47	562.63	2.27	12.77	253.29	54.18	9.39	2.01	2532.88	541.82	93.88	20.08	5389.11	1152.81	199.74	42.73	
Sample 120061	0.12	285.55	38.23	0.31	459.08	0.15	0.71	173.53	38.94	6.43	1.44	1735.29	389.39	64.32	14.43	5597.73	1256.09	207.48	46.56	F
Sample 120062	0.13	309.34	41.41	0.32	503.21	1.92	9.64	193.86	51.06	7.19	1.89	1938.64	510.56	71.85	18.92	6058.25	1595.51	224.55	59.14	
Sample 120063	0.11	261.75	35.04	0.27	294.41	1.04	3.05	32.66	38.09	1.21	1.41	326.59	380.92	12.10	14.12	1209.58	1410.83	44.83	52.29	M
Sample 120064	0.13	309.34	41.41	0.22	263.98	0.63	1.66	-45.37	43.08	-1.68	1.60	-453.67	430.76	-16.82	15.97	-2062.14	1957.98	-76.43	72.57	
Sample 120065	0.12	285.55	38.23	0.30	338.46	0.26	0.87	52.91	39.10	1.96	1.45	529.14	391.01	19.61	14.49	1763.79	1303.37	65.37	48.31	M
Sample 120066	0.13	309.34	41.41	0.35	388.85	0.75	2.92	79.51	44.33	2.95	1.64	795.09	443.34	29.47	16.43	2271.69	1266.68	84.20	46.95	
Sample 120067	0.11	261.75	35.04	0.17	447.19	0.60	2.70	185.44	37.74	6.87	1.40	1854.42	377.40	68.73	13.99	10908.34	2219.99	404.31	82.28	M
Sample 120068	0.13	309.34	41.41	0.16	315.26	0.07	0.23	5.92	41.64	0.22	1.54	59.21	416.43	2.19	15.43	370.08	2602.66	13.72	96.47	

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	Cotton Wool Mass (g)	Al^{3+} Conc CW (ng/mL)	SD	Mucus Mass (g)	Al^{3+}Conc ng/mL	%RSD	SD	Sample-CW ng/mL	SD	Sample-CW umol/ml	SD umol/ml	Al^{3+} Sample-CW ng/mL X10	Al^{3+} SD ng/mL X10	Al^{3+} Sample-CW umol/L X10	Al^{3+} SD umol/ml X10	Al^{3+} Sample-CW ng/mL per g mucus	Al^{3+} SD ng/mL per g mucus	Al^{3+} Sample-CW umol/L per g mucus	Al^{3+} SD umol/L per g mucus
Sample 120069	0.14	333.14	44.60	0.54	353.30	3.20	11.30	20.16	55.90	0.75	2.07	201.59	558.96	7.47	20.72	373.31	1035.12	13.84	38.37
Sample 120070	0.13	309.34	41.41	0.60	28554.27	1.69	483.74	28244.93	525.16	1046.88	19.46	282449.30	5251.57	10468.84	194.65	470748.83	8752.61	17448.07	324.41
Sample 120071	0.12	285.55	38.23	0.15	533.59	0.15	0.80	248.04	39.03	9.19	1.45	2480.42	390.26	91.94	14.46	16536.14	2601.71	612.90	96.43
Sample 120072	0.11	261.75	35.04	0.14	461.71	2.98	13.74	199.95	48.78	7.41	1.81	1999.53	487.84	74.11	18.08	14282.39	3484.59	529.37	129.15
Sample 120073	0.13	309.34	41.41	0.14	1082.41	1.94	20.99	773.07	62.40	28.65	2.31	7730.68	623.99	286.53	23.13	55219.12	4457.06	2046.67	165.20
Sample 120074	0.13	309.34	41.41	0.19	1287.84	3.53	45.47	978.50	86.89	36.27	3.22	9784.99	868.87	362.68	32.20	51499.97	4573.00	1908.82	169.50
Sample 120075	0.13	309.34	41.41	0.32	1804.05	0.74	13.35	1494.71	54.76	55.40	2.03	14947.08	547.60	554.01	20.30	46709.62	1711.24	1731.27	63.43
Sample 120076	0.13	309.34	41.41	0.36	1203.18	0.18	2.21	893.83	43.62	33.13	1.62	8938.32	436.20	331.29	16.17	24828.67	1211.66	920.26	44.91
Sample 120077	0.11	261.75	35.04	0.43	1063.77	1.01	10.76	802.02	45.80	29.73	1.70	8020.17	458.05	297.26	16.98	18651.55	1065.23	691.31	39.48
Sample 120078	0.12	285.55	38.23	0.47	1359.52	1.25	16.98	1073.97	55.21	39.81	2.05	10739.71	552.08	398.06	20.46	22850.44	1174.63	846.94	43.54
Sample 120079	0.12	285.55	38.23	0.57	570.08	3.95	22.54	284.53	60.77	10.55	2.25	2845.35	607.70	105.46	22.52	4991.84	1066.14	185.02	39.52
Sample 120080	0.12	285.55	38.23	0.61	1077.44	1.84	19.78	791.89	58.01	29.35	2.15	7918.91	580.08	293.51	21.50	12981.83	950.95	481.16	35.25
Sample 120081	0.12	285.55	38.23	0.58	582.17	3.70	21.56	296.62	59.79	10.99	2.22	2966.23	597.87	109.94	22.16	5114.19	1030.81	189.55	38.21
Sample 120082	0.12	285.55	38.23	1.07	755.54	2.39	18.02	469.99	56.25	17.42	2.08	4699.89	562.52	174.20	20.85	4392.42	525.72	162.80	19.49
Sample 120083	0.12	285.55	38.23	0.51	536.97	0.70	3.77	251.42	41.99	9.32	1.56	2514.21	419.94	93.19	15.57	4929.82	823.42	182.72	30.52
Sample 120084	0.12	285.55	38.23	0.45	391.23	1.79	6.99	105.68	45.22	3.92	1.68	1056.81	452.16	39.17	16.76	2348.47	1004.81	87.04	37.24
Sample 120085	0.12	285.55	38.23	0.20	289.70	2.16	6.26	4.15	44.49	0.15	1.65	41.49	444.88	1.54	16.49	207.43	2224.42	7.69	82.45
Sample 120086	0.13	309.34	41.41	0.18	328.26	0.21	0.68	18.92	42.09	0.70	1.56	189.16	420.91	7.01	15.60	1050.88	2338.36	38.95	86.67
Sample 120087	0.11	261.75	35.04	0.29	303.72	0.11	0.33	41.97	35.38	1.56	1.31	419.72	353.77	15.56	13.11	1447.32	1219.89	53.64	45.21
Sample 120088	0.13	309.34	41.41	0.33	325.04	2.24	7.28	15.69	48.69	0.58	1.80	156.95	486.93	5.82	18.05	475.60	1475.56	17.63	54.69
Sample 120089	0.13	309.34	41.41	0.41	609.04	1.93	11.78	299.69	53.19	11.11	1.97	2996.93	531.92	111.08	19.72	7309.60	1297.37	270.93	48.09
Sample 120090	0.12	285.55	38.23	0.41	366.93	1.18	4.33	81.38	42.56	3.02	1.58	813.81	425.59	30.16	15.77	1984.90	1038.04	73.57	38.47
Sample 120091	0.12	285.55	38.23	0.32	279.07	0.89	2.49	-6.47	40.72	-0.24	1.51	-64.74	407.16	-2.40	15.09	-202.31	1272.39	-7.50	47.16
Sample 120092	0.13	309.34	41.41	0.43	346.50	0.14	0.48	37.16	41.89	1.38	1.55	371.57	418.91	13.77	15.53	864.11	974.21	32.03	36.11
Sample 120093	0.12	285.55	38.23	0.17	294.72	1.48	4.36	9.17	42.59	0.34	1.58	91.68	425.93	3.40	15.79	539.30	2505.44	19.99	92.86
Sample 120094	0.12	285.55	38.23	0.30	327.50	1.23	4.02	41.95	42.25	1.55	1.57	419.53	422.48	15.55	15.66	1398.42	1408.26	51.83	52.20

(b) Cu^{2+}

	Cotton Wool Mass (g)	Cu^{2+} Conc CW (ng/mL)	SD	Mucus Mass (g)	Cu^{2+} Conc ng/mL	%RSD	SD	Sample-CW ng/mL	SD	Sample-CW umol/ml	SD umol/ml	Cu^{2+} Sample-CW ng/mL X10	Cu^{2+} SD ng/mL X10	Cu^{2+} Sample-CW umol/L X10	Cu^{2+} SD umol/ml X10	Cu^{2+} Sample-CW ng/mL per g mucus	Cu^{2+} SD ng/mL per g mucus	Cu^{2+} Sample-CW umol/L per g mucus	Cu^{2+} SD umol/L per g mucus	Sex
Sample 120015	0.15	7.49	1.22	0.38	18.66	1.41	0.26	11.17	1.48	0.17	0.02	111.70	14.81	1.73	0.23	293.94	38.97	4.55	0.60	F
Sample 120016	0.10	5.00	0.81	0.38	19.26	2.13	0.41	14.26	1.22	0.22	0.02	142.63	12.23	2.21	0.19	375.33	32.18	5.81	0.50	
Sample 120017	0.13	6.49	1.06	0.74	17.86	1.96	0.35	11.37	1.40	0.18	0.02	113.68	14.05	1.76	0.22	153.62	18.99	2.38	0.29	F
Sample 120018	0.10	5.00	0.81	0.75	23.23	0.52	0.12	18.23	0.93	0.28	0.01	182.33	9.32	2.82	0.14	243.11	12.43	3.77	0.19	
Sample 120019	0.12	5.99	0.97	0.39	24.17	1.44	0.35	18.17	1.32	0.28	0.02	181.72	13.23	2.82	0.20	465.96	33.92	7.22	0.53	M
Sample 120020	0.22	10.99	1.79	0.33	30.31	1.34	0.41	19.32	2.19	0.30	0.03	193.24	21.94	2.99	0.34	585.57	66.48	9.07	1.03	
Sample 120021	0.13	6.49	1.06	0.39	16.02	0.99	0.16	9.53	1.21	0.15	0.02	95.27	12.15	1.48	0.19	244.29	31.14	3.78	0.48	M
Sample 120022	0.13	6.49	1.06	0.46	22.61	0.38	0.09	16.12	1.14	0.25	0.02	161.18	11.42	2.50	0.18	350.38	24.82	5.43	0.38	
Sample 120023	0.11	5.49	0.89	0.33	26.78	1.62	0.43	21.29	1.33	0.33	0.02	212.86	13.27	3.30	0.21	645.04	40.21	9.99	0.62	F
Sample 120024	0.14	6.99	1.14	0.33	28.12	0.99	0.28	21.13	1.42	0.33	0.02	211.30	14.15	3.27	0.22	640.30	42.88	9.92	0.66	
Sample 120025	0.11	5.49	0.89	0.23	19.76	0.24	0.05	14.27	0.94	0.22	0.01	142.68	9.42	2.21	0.15	620.34	40.94	9.61	0.63	M
Sample 120026	0.11	5.49	0.89	0.31	22.24	5.10	1.13	16.75	2.03	0.26	0.03	167.46	20.27	2.59	0.31	540.20	65.39	8.37	1.01	
Sample 120027	0.12	5.99	0.97	0.62	41.87	0.28	0.12	35.88	1.09	0.56	0.02	358.79	10.92	5.56	0.17	578.69	17.61	8.97	0.27	M
Sample 120028	0.13	6.49	1.06	0.51	29.05	0.93	0.27	22.56	1.33	0.35	0.02	225.59	13.26	3.50	0.21	442.34	26.00	6.85	0.40	
Sample 120029	0.12	5.99	0.97	0.39	27.75	1.44	0.40	21.76	1.37	0.34	0.02	217.57	13.73	3.37	0.21	557.88	35.21	8.64	0.55	F
Sample 120030	0.12	5.99	0.97	0.32	22.20	0.99	0.22	16.21	1.19	0.25	0.02	162.09	11.94	2.51	0.19	506.52	37.32	7.85	0.58	
Sample 120031	0.12	5.99	0.97	0.18	17.25	1.76	0.30	11.26	1.28	0.17	0.02	112.56	12.77	1.74	0.20	625.36	70.96	9.69	1.10	M
Sample 120032	0.13	6.49	1.06	0.20	14.39	2.55	0.37	7.90	1.42	0.12	0.02	79.00	14.23	1.22	0.22	395.02	71.16	6.12	1.10	
Sample 120033	0.14	6.99	1.14	0.27	18.22	4.40	0.80	11.23	1.94	0.17	0.03	112.32	19.39	1.74	0.30	416.00	71.82	6.44	1.11	F
Sample 120034	0.12	5.99	0.97	0.22	18.49	1.33	0.25	12.50	1.22	0.19	0.02	124.98	12.20	1.94	0.19	568.10	55.46	8.80	0.86	
Sample 120035	0.13	6.49	1.06	0.50	24.35	2.32	0.57	17.86	1.62	0.28	0.03	178.59	16.22	2.77	0.25	357.18	32.43	5.53	0.50	F
Sample 120036	0.12	5.99	0.97	0.37	20.20	1.11	0.22	14.21	1.20	0.22	0.02	142.10	11.98	2.20	0.19	384.05	32.37	5.95	0.50	
Sample 120037	0.13	6.49	1.06	0.29	18.87	0.32	0.06	12.37	1.12	0.19	0.02	123.72	11.16	1.92	0.17	426.63	38.48	6.61	0.60	F
Sample 120038	0.12	5.99	0.97	0.21	15.00	5.32	0.80	9.01	1.77	0.14	0.03	90.06	17.72	1.40	0.27	428.83	84.38	6.64	1.31	
Sample 120039	0.12	5.99	0.97	0.20	17.19	0.54	0.09	11.20	1.07	0.17	0.02	111.97	10.68	1.73	0.17	559.83	53.39	8.67	0.83	M
Sample 120040	0.13	6.49	1.06	0.17	16.92	3.58	0.61	10.43	1.66	0.16	0.03	104.28	16.62	1.62	0.26	613.41	97.74	9.50	1.51	
Sample 120041	0.12	5.99	0.97	0.32	16.96	0.08	0.01	10.97	0.99	0.17	0.02	109.71	9.88	1.70	0.15	342.83	30.87	5.31	0.48	F

Cu^{2+} Page 2

	Cotton Wool Mass (g)	Cu^{2+} Conc CW (ng/mL)	SD	Mucus Mass (g)	Cu^{2+} Conc ng/mL	%RSD	SD	Sample-CW ng/mL	SD	Sample-CW umol/ml	SD umol/ml	Cu^{2+} Sample-CW ng/mL X10	Cu^{2+} SD ng/mL X10	Cu^{2+} Sample-CW umol/L X10	Cu^{2+} SD umol/ml X10	Cu^{2+} Sample-CW ng/mL per g mucus	Cu^{2+} SD ng/mL per g mucus	Cu^{2+} Sample-CW umol/L per g mucus	Cu^{2+} SD umol/L per g mucus	Sex
Sample 120042	0.13	6.49	1.06	0.21	15.59	2.18	0.34	9.10	1.39	0.14	0.02	90.98	13.95	1.41	0.22	433.23	66.42	6.71	1.03	
Sample 120043	0.15	7.49	1.22	0.20	17.02	2.80	0.48	9.53	1.69	0.15	0.03	95.26	16.95	1.48	0.26	476.30	84.74	7.38	1.31	F
Sample 120044	0.14	6.99	1.14	0.18	17.30	1.00	0.17	10.31	1.31	0.16	0.02	103.11	13.10	1.60	0.20	572.84	72.76	8.87	1.13	
Sample 120045	0.12	5.99	0.97	0.16	20.33	0.54	0.11	14.34	1.08	0.22	0.02	143.39	10.84	2.22	0.17	896.17	67.78	13.88	1.05	F
Sample 120046	0.14	6.99	1.14	0.10	13.54	0.53	0.07	6.55	1.21	0.10	0.02	65.47	12.09	1.01	0.19	654.74	120.88	10.14	1.87	
Sample 120047	0.12	5.99	0.97	0.20	14.03	4.24	0.60	8.04	1.57	0.12	0.02	80.35	15.70	1.24	0.24	401.77	78.48	6.22	1.22	M
Sample 120048	0.13	6.49	1.06	0.12	11.52	0.82	0.09	5.03	1.15	0.08	0.02	50.30	11.51	0.78	0.18	419.17	95.89	6.49	1.49	
Sample 120049	0.11	5.49	0.89	0.22	15.32	0.19	0.03	9.83	0.92	0.15	0.01	98.27	9.22	1.52	0.14	446.67	41.92	6.92	0.65	F
Sample 120050	0.13	6.49	1.06	0.40	18.11	0.39	0.07	11.61	1.13	0.18	0.02	116.14	11.27	1.80	0.17	290.36	28.16	4.50	0.44	
Sample 120051	0.11	5.49	0.89	0.15	17.27	2.67	0.46	11.78	1.35	0.18	0.02	117.76	13.54	1.82	0.21	785.06	90.26	12.16	1.40	M
Sample 120052	0.12	5.99	0.97	0.15	25.62	4.19	1.07	19.62	2.05	0.30	0.03	196.23	20.49	3.04	0.32	1308.22	136.60	20.27	2.12	
Sample 120053	0.14	6.99	1.14	0.14	24.82	0.48	0.12	17.82	1.26	0.28	0.02	178.22	12.55	2.76	0.19	1273.03	89.64	19.72	1.39	M
Sample 120054	0.13	6.49	1.06	0.36	32.22	3.88	1.25	25.73	2.30	0.40	0.04	257.28	23.05	3.99	0.36	714.66	64.01	11.07	0.99	
Sample 120055	0.12	5.99	0.97	0.40	13.74	0.68	0.09	7.74	1.07	0.12	0.02	77.45	10.68	1.20	0.17	193.62	26.70	3.00	0.41	F
Sample 120056	0.13	6.49	1.06	0.34	16.35	0.49	0.08	9.86	1.14	0.15	0.02	98.61	11.35	1.53	0.18	290.04	33.38	4.49	0.52	
Sample 120057	0.12	5.99	0.97	0.37	17.33	2.18	0.38	11.34	1.35	0.18	0.02	113.39	13.52	1.76	0.21	306.45	36.54	4.75	0.57	M
Sample 120058	0.13	6.49	1.06	0.52	20.97	3.08	0.65	14.47	1.70	0.22	0.03	144.73	17.02	2.24	0.26	278.32	32.73	4.31	0.51	
Sample 120059	0.13	6.49	1.06	0.43	22.35	3.15	0.70	15.86	1.76	0.25	0.03	158.60	17.60	2.46	0.27	368.84	40.93	5.71	0.63	M
Sample 120060	0.13	6.49	1.06	0.47	27.18	3.51	0.95	20.69	2.01	0.32	0.03	206.87	20.10	3.21	0.31	440.16	42.77	6.82	0.66	
Sample 120061	0.12	5.99	0.97	0.31	15.59	0.38	0.06	9.60	1.03	0.15	0.02	95.97	10.34	1.49	0.16	309.58	33.36	4.80	0.52	F
Sample 120062	0.13	6.49	1.06	0.32	19.39	2.14	0.41	12.89	1.47	0.20	0.02	128.92	14.71	2.00	0.23	402.86	45.96	6.24	0.71	
Sample 120063	0.11	5.49	0.89	0.27	14.52	1.08	0.16	9.02	1.05	0.14	0.02	90.21	10.51	1.40	0.16	334.12	38.91	5.18	0.60	M
Sample 120064	0.13	6.49	1.06	0.22	16.15	1.94	0.31	9.66	1.37	0.15	0.02	96.59	13.69	1.50	0.21	439.06	62.22	6.80	0.96	
Sample 120065	0.12	5.99	0.97	0.30	32.75	2.17	0.71	26.76	1.69	0.41	0.03	267.60	16.85	4.15	0.26	891.98	56.17	13.82	0.87	M
Sample 120066	0.13	6.49	1.06	0.35	36.87	0.98	0.36	30.38	1.42	0.47	0.02	303.79	14.16	4.71	0.22	867.96	40.46	13.45	0.63	
Sample 120067	0.11	5.49	0.89	0.17	24.84	1.15	0.28	19.34	1.18	0.30	0.02	193.42	11.78	3.00	0.18	1137.77	69.31	17.63	1.07	M
Sample 120068	0.13	6.49	1.06	0.16	18.21	0.06	0.01	11.72	1.07	0.18	0.02	117.16	10.66	1.82	0.17	732.27	66.64	11.34	1.03	

Cu^{2+} Page 3

	Cotton Wool Mass (g)	Cu^{2+} Conc CW (ng/mL)	SD	Mucus Mass (g)	Cu^{2+} Conc ng/mL	%RSD	SD	Sample-CW ng/mL	SD	Sample-CW umol/ml	SD umol/ml	Cu^{2+} Sample-CW ng/mL X10	Cu^{2+} SD ng/mL X10	Cu^{2+} Sample-CW umol/L X10	Cu^{2+} SD umol/ml X10	Cu^{2+} Sample-CW ng/mL per g mucus	Cu^{2+} SD ng/mL per g mucus	Cu^{2+} Sample-CW umol/L per g mucus	Cu^{2+} SD umol/L per g mucus	Sex
Sample 120069	0.14	6.99	1.14	0.54	16.54	1.78	0.30	9.55	1.43	0.15	0.02	95.46	14.32	1.48	0.22	176.78	26.52	2.74	0.41	F
Sample 120070	0.13	6.49	1.06	0.60	58.02	0.71	0.41	51.52	1.47	0.80	0.02	515.22	14.68	7.98	0.23	858.69	24.46	13.30	0.38	
Sample 120071	0.12	5.99	0.97	0.15	21.46	1.09	0.23	15.47	1.21	0.24	0.02	154.67	12.09	2.40	0.19	1031.15	80.59	15.98	1.25	F
Sample 120072	0.11	5.49	0.89	0.14	18.82	0.66	0.12	13.33	1.02	0.21	0.02	133.25	10.18	2.06	0.16	951.79	72.69	14.75	1.13	
Sample 120073	0.13	6.49	1.06	0.14	19.12	2.50	0.48	12.62	1.53	0.20	0.02	126.23	15.34	1.96	0.24	901.65	109.56	13.97	1.70	M
Sample 120074	0.13	6.49	1.06	0.19	19.27	2.12	0.41	12.78	1.46	0.20	0.02	127.77	14.64	1.98	0.23	672.46	77.03	10.42	1.19	
Sample 120075	0.13	6.49	1.06	0.32	11.90	0.10	0.01	5.41	1.07	0.08	0.02	54.11	10.67	0.84	0.17	169.09	33.35	2.62	0.52	F
Sample 120076	0.13	6.49	1.06	0.36	14.59	0.89	0.13	8.09	1.19	0.13	0.02	80.93	11.85	1.25	0.18	224.79	32.92	3.48	0.51	
Sample 120077	0.11	5.49	0.89	0.43	16.77	2.53	0.42	11.27	1.32	0.17	0.02	112.72	13.18	1.75	0.20	262.14	30.64	4.06	0.47	F
Sample 120078	0.12	5.99	0.97	0.47	15.55	0.07	0.01	9.56	0.99	0.15	0.02	95.61	9.86	1.48	0.15	203.42	20.97	3.15	0.32	
Sample 120079	0.12	5.99	0.97	0.57	15.71	2.16	0.34	9.71	1.31	0.15	0.02	97.15	13.14	1.51	0.20	170.44	23.06	2.64	0.36	M
Sample 120080	0.12	5.99	0.97	0.61	14.42	2.81	0.40	8.43	1.38	0.13	0.02	84.31	13.79	1.31	0.21	138.21	22.61	2.14	0.35	
Sample 120081	0.12	5.99	0.97	0.58	12.11	1.20	0.15	6.12	1.12	0.09	0.02	61.19	11.20	0.95	0.17	105.51	19.31	1.63	0.30	M
Sample 120082	0.12	5.99	0.97	1.07	25.22	1.94	0.49	19.22	1.46	0.30	0.02	192.24	14.63	2.98	0.23	179.66	13.67	2.78	0.21	
Sample 120083	0.12	5.99	0.97	0.51	13.42	0.36	0.05	7.42	1.02	0.12	0.02	74.23	10.23	1.15	0.16	145.55	20.05	2.25	0.31	M
Sample 120084	0.12	5.99	0.97	0.45	18.16	0.72	0.13	12.17	1.10	0.19	0.02	121.67	11.05	1.88	0.17	270.37	24.55	4.19	0.38	
Sample 120085	0.12	5.99	0.97	0.20	14.48	1.24	0.18	8.48	1.15	0.13	0.02	84.81	11.55	1.31	0.18	424.05	57.73	6.57	0.89	F
Sample 120086	0.13	6.49	1.06	0.18	19.79	2.97	0.59	13.30	1.64	0.21	0.03	133.00	16.43	2.06	0.25	738.89	91.30	11.45	1.41	
Sample 120087	0.11	5.49	0.89	0.29	30.89	1.41	0.43	25.39	1.33	0.39	0.02	253.94	13.28	3.93	0.21	875.65	45.79	13.57	0.71	M
Sample 120088	0.13	6.49	1.06	0.33	13.99	1.81	0.25	7.49	1.31	0.12	0.02	74.95	13.09	1.16	0.20	227.11	39.66	3.52	0.61	
Sample 120089	0.13	6.49	1.06	0.41	20.26	4.79	0.97	13.77	2.03	0.21	0.03	137.66	20.25	2.13	0.31	335.76	49.40	5.20	0.77	M
Sample 120090	0.12	5.99	0.97	0.41	17.82	0.17	0.03	11.82	1.00	0.18	0.02	118.22	10.05	1.83	0.16	288.35	24.51	4.47	0.38	
Sample 120091	0.12	5.99	0.97	0.32	11.93	0.07	0.01	5.94	0.98	0.09	0.02	59.40	9.83	0.92	0.15	185.63	30.71	2.88	0.48	F
Sample 120092	0.13	6.49	1.06	0.43	15.63	6.64	1.04	9.13	2.09	0.14	0.03	91.33	20.94	1.41	0.32	212.39	48.70	3.29	0.75	
Sample 120093	0.12	5.99	0.97	0.17	12.03	0.81	0.10	6.04	1.07	0.09	0.02	60.39	10.73	0.94	0.17	355.23	63.09	5.50	0.98	F
Sample 120094	0.12	5.99	0.97	0.30	13.42	0.59	0.08	7.42	1.05	0.11	0.02	74.22	10.54	1.15	0.16	247.42	35.13	3.83	0.54	

(c) Fe^{3+}

	Cotton Wool Mass (g)	Fe^{3+} Conc CW (ng/mL)	SD	Mucus Mass (g)	Fe^{3+} Conc ng/mL	%RSD	SD	Sample-CW ng/mL	SD	Sample-CW umol/ml	SD umol/ml	Fe^{3+} Sample-CW ng/mL X10	Fe^{3+} SD ng/mL X10	Fe^{3+} Sample-CW umol/L X10	Fe^{3+} SD umol/ml X10	Fe^{3+} Sample-CW ng/mL per g mucus	Fe^{3+} SD ng/mL per g mucus	Fe^{3+} Sample-CW umol/L per g mucus	Fe^{3+} SD umol/L per g mucus	Sex
Sample 120015	0.15	266.25	14.13	0.38	279.83	0.89	2.48	13.58	16.62	0.24	0.30	135.79	166.16	2.43	2.98	357.35	437.27	6.40	7.83	F
Sample 120016	0.10	177.50	9.42	0.38	302.95	0.68	2.07	125.45	11.49	2.25	0.21	1254.46	114.89	22.46	2.06	3301.22	302.34	59.11	5.41	
Sample 120017	0.13	230.75	12.25	0.74	377.73	1.77	6.68	146.98	18.92	2.63	0.34	1469.75	189.24	26.32	3.39	1986.15	255.73	35.57	4.58	F
Sample 120018	0.10	177.50	9.42	0.75	462.10	0.44	2.03	284.60	11.45	5.10	0.21	2845.98	114.51	50.96	2.05	3794.64	152.68	67.95	2.73	
Sample 120019	0.12	213.00	11.31	0.39	314.23	0.39	1.23	101.23	12.54	1.81	0.22	1012.31	125.39	18.13	2.25	2595.68	321.51	46.48	5.76	M
Sample 120020	0.22	390.51	20.73	0.33	432.66	1.30	5.64	42.16	26.36	0.75	0.47	421.60	263.63	7.55	4.72	1277.56	798.88	22.88	14.31	
Sample 120021	0.13	230.75	12.25	0.39	341.58	2.01	6.87	110.83	19.12	1.98	0.34	1108.29	191.20	19.85	3.42	2841.76	490.26	50.89	8.78	M
Sample 120022	0.13	230.75	12.25	0.46	411.02	3.90	16.05	180.27	28.29	3.23	0.51	1802.69	282.94	32.28	5.07	3918.90	615.08	70.17	11.01	
Sample 120023	0.11	195.25	10.36	0.33	409.88	1.05	4.31	214.63	14.67	3.84	0.26	2146.29	146.74	38.43	2.63	6503.90	444.66	116.46	7.96	F
Sample 120024	0.14	248.50	13.19	0.33	518.39	1.82	9.42	269.88	22.61	4.83	0.40	2698.83	226.07	48.33	4.05	8178.27	685.06	146.45	12.27	
Sample 120025	0.11	195.25	10.36	0.23	301.16	0.23	0.70	105.91	11.06	1.90	0.20	1059.07	110.61	18.96	1.98	4604.64	480.89	82.45	8.61	M
Sample 120026	0.11	195.25	10.36	0.31	333.23	0.78	2.59	137.98	12.96	2.47	0.23	1379.81	129.55	24.71	2.32	4451.02	417.91	79.70	7.48	
Sample 120027	0.12	213.00	11.31	0.62	355.06	1.24	4.41	142.05	15.72	2.54	0.28	1420.54	157.20	25.44	2.81	2291.20	253.55	41.03	4.54	M
Sample 120028	0.13	230.75	12.25	0.51	444.95	0.08	0.37	214.20	12.62	3.84	0.23	2141.96	126.17	38.36	2.26	4199.92	247.38	75.21	4.43	
Sample 120029	0.12	213.00	11.31	0.39	370.85	1.04	3.85	157.85	15.16	2.83	0.27	1578.46	151.58	28.27	2.71	4047.33	388.67	72.47	6.96	F
Sample 120030	0.12	213.00	11.31	0.32	371.61	0.00	0.02	158.61	11.32	2.84	0.20	1586.07	113.21	28.40	2.03	4956.48	353.79	88.75	6.34	
Sample 120031	0.12	213.00	11.31	0.18	374.50	3.19	11.93	161.50	23.24	2.89	0.42	1614.96	232.36	28.92	4.16	8971.98	1290.89	160.66	23.12	M
Sample 120032	0.13	230.75	12.25	0.20	414.29	1.74	7.21	183.54	19.46	3.29	0.35	1835.36	194.60	32.87	3.48	9176.78	972.99	164.33	17.42	
Sample 120033	0.14	248.50	13.19	0.27	441.64	1.10	4.87	193.14	18.06	3.46	0.32	1931.40	180.60	34.59	3.23	7153.35	668.89	128.09	11.98	F
Sample 120034	0.12	213.00	11.31	0.22	356.82	1.07	3.82	143.81	15.12	2.58	0.27	1438.14	151.25	25.75	2.71	6537.02	687.50	117.06	12.31	
Sample 120035	0.13	230.75	12.25	0.50	586.95	0.56	3.26	356.19	15.51	6.38	0.28	3561.93	155.07	63.78	2.78	7123.86	310.15	127.56	5.55	F
Sample 120036	0.12	213.00	11.31	0.37	377.05	1.53	5.75	164.04	17.06	2.94	0.31	1640.42	170.57	29.37	3.05	4433.58	460.99	79.39	8.25	
Sample 120037	0.13	230.75	12.25	0.29	441.57	0.38	1.66	210.82	13.91	3.78	0.25	2108.15	139.07	37.75	2.49	7269.49	479.54	130.17	8.59	F
Sample 120038	0.12	213.00	11.31	0.21	342.44	0.97	3.32	129.44	14.63	2.32	0.26	1294.39	146.30	23.18	2.62	6163.75	696.66	110.37	12.47	
Sample 120039	0.12	213.00	11.31	0.20	365.66	1.10	4.01	152.66	15.31	2.73	0.27	1526.56	153.14	27.34	2.74	7632.78	765.69	136.68	13.71	M
Sample 120040	0.13	230.75	12.25	0.17	348.34	1.10	3.85	117.59	16.09	2.11	0.29	1175.90	160.94	21.06	2.88	6917.04	946.68	123.86	16.95	
Sample 120041	0.12	213.00	11.31	0.32	365.76	2.24	8.18	152.76	19.49	2.74	0.35	1527.62	194.86	27.35	3.49	4773.82	608.93	85.48	10.90	F

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	Cotton Wool Mass (g)	Fe^{3+} Conc CW (ng/mL)	SD	Mucus Mass (g)	Fe^{3+}Conc ng/mL	%RSD	SD	Sample-CW ng/mL	SD	Sample-CW umol/ml	SD umol/ml	Fe^{3+} Sample-CW ng/mL X10	Fe^{3+} SD ng/mL X10	Fe^{3+} Sample-CW umol/L X10	Fe^{3+} SD umol/ml X10	Fe^{3+} Sample-CW ng/mL per g mucus	Fe^{3+} SD ng/mL per g mucus	Fe^{3+} Sample-CW umol/L per g mucus	Fe^{3+} SD umol/L per g mucus	Sex
Sample 120042	0.13	230.75	12.25	0.21	380.30	0.70	2.65	149.55	14.90	2.68	0.27	1495.50	148.98	26.78	2.67	7121.45	709.45	127.52	12.70	
Sample 120043	0.15	266.25	14.13	0.20	498.19	1.94	9.68	231.94	23.81	4.15	0.43	2319.36	238.08	41.53	4.26	11596.82	1190.42	207.66	21.32	F
Sample 120044	0.14	248.50	13.19	0.18	464.85	2.35	10.93	216.35	24.12	3.87	0.43	2163.45	241.22	38.74	4.32	12019.19	1340.10	215.22	24.00	
Sample 120045	0.12	213.00	11.31	0.16	368.14	1.07	3.96	155.14	15.26	2.78	0.27	1551.42	152.62	27.78	2.73	9696.36	953.87	173.63	17.08	F
Sample 120046	0.14	248.50	13.19	0.10	458.67	1.53	7.00	210.17	20.19	3.76	0.36	2101.66	201.93	37.63	3.62	21016.60	2019.31	376.34	36.16	
Sample 120047	0.12	213.00	11.31	0.20	353.58	1.38	4.88	140.57	16.19	2.52	0.29	1405.74	161.85	25.17	2.90	7028.72	809.25	125.86	14.49	M
Sample 120048	0.13	230.75	12.25	0.12	345.43	1.90	6.56	114.68	18.81	2.05	0.34	1146.81	188.09	20.54	3.37	9556.76	1567.41	171.13	28.07	
Sample 120049	0.11	195.25	10.36	0.22	368.08	0.01	0.03	172.82	10.39	3.09	0.19	1728.24	103.94	30.95	1.86	7855.63	472.45	140.67	8.46	F
Sample 120050	0.13	230.75	12.25	0.40	368.64	2.27	8.37	137.88	20.62	2.47	0.37	1378.83	206.22	24.69	3.69	3447.07	515.54	61.73	9.23	
Sample 120051	0.11	195.25	10.36	0.15	320.30	0.70	2.24	125.04	12.61	2.24	0.23	1250.43	126.07	22.39	2.26	8336.18	840.48	149.27	15.05	M
Sample 120052	0.12	213.00	11.31	0.15	337.57	2.23	7.52	124.57	18.82	2.23	0.34	1245.70	188.23	22.31	3.37	8304.66	1254.86	148.71	22.47	
Sample 120053	0.14	248.50	13.19	0.14	362.98	0.04	0.13	114.47	13.32	2.05	0.24	1144.73	133.17	20.50	2.38	8176.63	951.25	146.42	17.03	M
Sample 120054	0.13	230.75	12.25	0.36	381.62	0.76	2.91	150.86	15.16	2.70	0.27	1508.65	151.62	27.01	2.72	4190.68	421.17	75.04	7.54	
Sample 120055	0.12	213.00	11.31	0.40	437.66	0.87	3.80	224.66	15.11	4.02	0.27	2246.61	151.09	40.23	2.71	5616.53	377.72	100.57	6.76	F
Sample 120056	0.13	230.75	12.25	0.34	503.41	0.68	3.43	272.66	15.68	4.88	0.28	2726.59	156.81	48.82	2.81	8019.39	461.22	143.60	8.26	
Sample 120057	0.12	213.00	11.31	0.37	457.09	0.81	3.70	244.09	15.01	4.37	0.27	2440.89	150.05	43.71	2.69	6597.00	405.54	118.13	7.26	M
Sample 120058	0.13	230.75	12.25	0.52	407.91	0.32	1.30	177.16	13.55	3.17	0.24	1771.56	135.51	31.72	2.43	3406.85	260.60	61.01	4.67	
Sample 120059	0.13	230.75	12.25	0.43	427.50	0.45	1.94	196.75	14.19	3.52	0.25	1967.48	141.88	35.23	2.54	4575.53	329.96	81.93	5.91	M
Sample 120060	0.13	230.75	12.25	0.47	622.42	2.91	18.08	391.67	30.33	7.01	0.54	3916.65	303.32	70.13	5.43	8333.31	645.36	149.22	11.56	
Sample 120061	0.12	213.00	11.31	0.31	403.90	0.62	2.52	190.90	13.82	3.42	0.25	1908.96	138.21	34.18	2.47	6157.94	445.84	110.27	7.98	F
Sample 120062	0.13	230.75	12.25	0.32	537.03	3.09	16.62	306.28	28.86	5.48	0.52	3062.77	288.63	54.84	5.17	9571.17	901.95	171.39	16.15	
Sample 120063	0.11	195.25	10.36	0.27	444.93	2.54	11.28	249.68	21.64	4.47	0.39	2496.79	216.43	44.71	3.88	9247.37	801.60	165.59	14.35	M
Sample 120064	0.13	230.75	12.25	0.22	351.87	0.29	1.01	121.12	13.25	2.17	0.24	1211.19	132.53	21.69	2.37	5505.42	602.40	98.58	10.79	
Sample 120065	0.12	213.00	11.31	0.30	509.47	1.47	7.51	296.46	18.82	5.31	0.34	2964.64	188.18	53.09	3.37	9882.14	627.26	176.96	11.23	M
Sample 120066	0.13	230.75	12.25	0.35	372.91	0.23	0.85	142.16	13.10	2.55	0.23	1421.58	131.02	25.46	2.35	4061.65	374.34	72.73	6.70	
Sample 120067	0.11	195.25	10.36	0.17	366.61	1.94	7.11	171.36	17.47	3.07	0.31	1713.60	174.70	30.68	3.13	10080.00	1027.66	180.50	18.40	M
Sample 120068	0.13	230.75	12.25	0.16	379.36	0.06	0.24	148.60	12.48	2.66	0.22	1486.03	124.83	26.61	2.24	9287.69	780.17	166.31	13.97	

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	Cotton Wool Mass (g)	Fe^{3+} Conc CW (ng/mL)	SD	Mucus Mass (g)	Fe^{3+}Conc ng/mL	%RSD	SD	Sample-CW ng/mL	SD	Sample-CW umol/ml	SD umol/ml	Fe^{3+} Sample-CW ng/mL X10	Fe^{3+} SD ng/mL X10	Fe^{3+} Sample-CW umol/L X10	Fe^{3+} SD umol/ml X10	Fe^{3+} Sample-CW ng/mL per g mucus	Fe^{3+} SD ng/mL per g mucus	Fe^{3+} Sample-CW umol/L per g mucus	Fe^{3+} SD umol/L per g mucus	Sex
Sample 120069	0.14	248.50	13.19	0.54	404.75	3.21	12.99	156.25	26.17	2.80	0.47	1562.52	261.75	27.98	4.69	2893.55	484.71	51.81	8.68	F
Sample 120070	0.13	230.75	12.25	0.60	375.82	2.20	8.27	145.07	20.52	2.60	0.37	1450.66	205.18	25.98	3.67	2417.76	341.96	43.29	6.12	
Sample 120071	0.12	213.00	11.31	0.15	421.23	0.41	1.73	208.23	13.03	3.73	0.23	2082.29	130.34	37.29	2.33	13881.91	868.90	248.58	15.56	F
Sample 120072	0.11	195.25	10.36	0.14	362.33	1.53	5.56	167.08	15.92	2.99	0.29	1670.82	159.22	29.92	2.85	11934.42	1137.28	213.71	20.36	
Sample 120073	0.13	230.75	12.25	0.14	508.77	1.57	7.97	278.02	20.22	4.98	0.36	2780.20	202.17	49.78	3.62	19858.57	1444.05	355.60	25.86	M
Sample 120074	0.13	230.75	12.25	0.19	400.38	4.55	18.21	169.63	30.45	3.04	0.55	1696.29	304.53	30.38	5.45	8927.86	1602.81	159.87	28.70	
Sample 120075	0.13	230.75	12.25	0.32	310.60	2.28	7.08	79.84	19.32	1.43	0.35	798.43	193.24	14.30	3.46	2495.09	603.87	44.68	10.81	F
Sample 120076	0.13	230.75	12.25	0.36	933.56	0.21	1.96	702.81	14.21	12.58	0.25	7028.06	142.07	125.85	2.54	19522.38	394.63	349.58	7.07	
Sample 120077	0.11	195.25	10.36	0.43	1184.70	1.61	19.13	989.44	29.49	17.72	0.53	9894.45	294.90	177.18	5.28	23010.35	685.82	412.04	12.28	F
Sample 120078	0.12	213.00	11.31	0.47	395.09	3.11	12.30	182.09	23.61	3.26	0.42	1820.90	236.07	32.61	4.23	3874.25	502.27	69.38	8.99	
Sample 120079	0.12	213.00	11.31	0.57	586.94	3.25	19.10	373.93	30.40	6.70	0.54	3739.35	304.02	66.96	5.44	6560.26	533.38	117.47	9.55	M
Sample 120080	0.12	213.00	11.31	0.61	322.08	3.01	9.69	109.08	21.00	1.95	0.38	1090.80	209.96	19.53	3.76	1788.20	344.19	32.02	6.16	
Sample 120081	0.12	213.00	11.31	0.58	291.05	3.29	9.56	78.04	20.87	1.40	0.37	780.43	208.70	13.97	3.74	1345.57	359.83	24.09	6.44	M
Sample 120082	0.12	213.00	11.31	1.07	431.16	2.68	11.57	218.16	22.87	3.91	0.41	2181.56	228.73	39.06	4.10	2038.85	213.77	36.51	3.83	
Sample 120083	0.12	248.50	13.19	0.51	361.17	1.08	3.91	112.67	17.10	2.02	0.31	1126.65	171.04	20.17	3.06	2209.12	335.37	39.56	6.01	M
Sample 120084	0.12	213.00	11.31	0.45	377.52	0.59	2.22	164.52	13.52	2.95	0.24	1645.16	135.23	29.46	2.42	3655.90	300.51	65.47	5.38	
Sample 120085	0.12	213.00	11.31	0.20	374.25	1.55	5.79	161.25	17.10	2.89	0.31	1612.47	170.96	28.87	3.06	8062.34	854.80	144.37	15.31	F
Sample 120086	0.13	230.75	12.25	0.18	408.93	2.52	10.29	178.18	22.54	3.19	0.40	1781.82	225.36	31.91	4.04	9899.00	1251.99	177.26	22.42	
Sample 120087	0.11	195.25	10.36	0.29	366.85	0.75	2.73	171.59	13.10	3.07	0.23	1715.94	130.97	30.73	2.35	5917.05	451.61	105.95	8.09	M
Sample 120088	0.13	230.75	12.25	0.33	310.32	2.34	7.25	79.56	19.50	1.42	0.35	795.65	195.02	14.25	3.49	2411.05	590.96	43.17	10.58	
Sample 120089	0.13	230.75	12.25	0.41	284.89	3.05	8.69	54.14	20.94	0.97	0.37	541.36	209.36	9.69	3.75	1320.40	510.62	23.64	9.14	M
Sample 120090	0.12	213.00	11.31	0.41	275.32	1.86	5.11	62.32	16.42	1.12	0.29	623.19	164.15	11.16	2.94	1519.99	400.37	27.22	7.17	
Sample 120091	0.12	213.00	11.31	0.32	249.63	0.72	1.80	36.63	13.10	0.66	0.23	366.31	131.00	6.56	2.35	1144.73	409.39	20.50	7.33	F
Sample 120092	0.13	230.75	12.25	0.43	270.43	1.78	4.81	39.68	17.06	0.71	0.31	396.78	170.58	7.11	3.05	922.75	396.69	16.52	7.10	
Sample 120093	0.12	213.00	11.31	0.17	267.15	1.59	4.24	54.15	15.54	0.97	0.28	541.48	155.40	9.70	2.78	3185.20	914.14	57.04	16.37	F
Sample 120094	0.12	213.00	11.31	0.30	313.00	1.17	3.66	100.00	14.96	1.79	0.27	1000.01	149.63	17.91	2.68	3333.36	498.77	59.69	8.93	

(d) Mn^{2+}

	Cotton Wool Mass (g)	Mn^{2+} Conc CW (ng/mL)	SD	Mucus Mass (g)	Mn^{2+}Conc ng/mL	%RSD	SD	Sample-CW ng/mL	SD	Sample-CW umol/ml	SD umol/ml	Mn^{2+} Sample-CW ng/mL X10	Mn^{2+} SD ng/mL X10	Mn^{2+} Sample-CW umol/L X10	Mn^{2+} SD umol/ml X10	Mn^{2+} Sample-CW ng/mL per g mucus	Mn^{2+} SD ng/mL per g mucus	Mn^{2+} Sample-CW umol/L per g mucus	Mn^{2+} SD umol/L per g mucus	Sex
Sample 120015	0.15	28.07	1.54	0.38	21.05	0.83	0.17	-7.02	1.71	-0.13	0.03	-70.17	17.11	-1.28	0.31	-184.66	45.02	-3.36	0.82	F
Sample 120016	0.10	18.71	1.02	0.38	19.03	0.12	0.02	0.32	1.05	0.01	0.02	3.19	10.47	0.06	0.19	8.40	27.55	0.15	0.50	
Sample 120017	0.13	24.33	1.33	0.74	18.04	1.17	0.21	-6.29	1.54	-0.11	0.03	-62.89	15.42	-1.14	0.28	-84.98	20.84	-1.55	0.38	F
Sample 120018	0.10	18.71	1.02	0.75	24.26	1.20	0.29	5.54	1.31	0.10	0.02	55.44	13.14	1.01	0.24	73.92	17.53	1.35	0.32	
Sample 120019	0.12	22.45	1.23	0.39	22.01	1.94	0.43	-0.45	1.66	-0.01	0.03	-4.47	16.57	-0.08	0.30	-11.47	42.48	-0.21	0.77	M
Sample 120020	0.22	41.17	2.25	0.33	25.52	1.61	0.41	-15.64	2.66	-0.28	0.05	-156.43	26.65	-2.85	0.49	-474.04	80.75	-8.63	1.47	
Sample 120021	0.13	24.33	1.33	0.39	26.73	1.93	0.52	2.41	1.85	0.04	0.03	24.05	18.48	0.44	0.34	61.67	47.38	1.12	0.86	M
Sample 120022	0.13	24.33	1.33	0.46	26.87	1.80	0.48	2.55	1.81	0.05	0.03	25.48	18.15	0.46	0.33	55.40	39.46	1.01	0.72	
Sample 120023	0.11	20.58	1.13	0.33	25.11	0.65	0.16	4.52	1.29	0.08	0.02	45.23	12.91	0.82	0.24	137.05	39.12	2.49	0.71	F
Sample 120024	0.14	26.20	1.43	0.33	31.54	0.52	0.16	5.34	1.60	0.10	0.03	53.41	15.97	0.97	0.29	161.85	48.40	2.95	0.88	
Sample 120025	0.11	20.58	1.13	0.23	16.70	0.43	0.07	-3.88	1.20	-0.07	0.02	-38.82	11.98	-0.71	0.22	-168.78	52.07	-3.07	0.95	M
Sample 120026	0.11	20.58	1.13	0.31	26.36	1.21	0.32	5.78	1.45	0.11	0.03	57.79	14.46	1.05	0.26	186.41	46.66	3.39	0.85	
Sample 120027	0.12	22.45	1.23	0.62	30.99	0.40	0.12	8.54	1.35	0.16	0.02	85.39	13.53	1.55	0.25	137.73	21.82	2.51	0.40	M
Sample 120028	0.13	24.33	1.33	0.51	30.72	1.00	0.31	6.39	1.64	0.12	0.03	63.90	16.39	1.16	0.30	125.29	32.14	2.28	0.59	
Sample 120029	0.12	22.45	1.23	0.39	28.42	0.32	0.09	5.97	1.32	0.11	0.02	59.66	13.21	1.09	0.24	152.98	33.88	2.78	0.62	F
Sample 120030	0.12	22.45	1.23	0.32	26.70	1.50	0.40	4.24	1.63	0.08	0.03	42.42	16.31	0.77	0.30	132.56	50.96	2.41	0.93	
Sample 120031	0.12	22.45	1.23	0.18	24.39	2.39	0.58	1.94	1.81	0.04	0.03	19.39	18.12	0.35	0.33	107.71	100.64	1.96	1.83	M
Sample 120032	0.13	24.33	1.33	0.20	26.58	0.57	0.15	2.26	1.48	0.04	0.03	22.58	14.82	0.41	0.27	112.90	74.10	2.06	1.35	
Sample 120033	0.14	26.20	1.43	0.27	28.47	0.85	0.24	2.28	1.68	0.04	0.03	22.76	16.77	0.41	0.31	84.29	62.12	1.53	1.13	F
Sample 120034	0.12	22.45	1.23	0.22	24.83	1.23	0.31	2.37	1.53	0.04	0.03	23.71	15.34	0.43	0.28	107.76	69.75	1.96	1.27	
Sample 120035	0.13	24.33	1.33	0.50	46.41	1.49	0.69	22.09	2.02	0.40	0.04	220.89	20.23	4.02	0.37	441.77	40.46	8.04	0.74	F
Sample 120036	0.12	22.45	1.23	0.37	24.69	1.53	0.38	2.24	1.61	0.04	0.03	22.37	16.06	0.41	0.29	60.47	43.41	1.10	0.79	
Sample 120037	0.13	24.33	1.33	0.29	26.73	1.56	0.42	2.41	1.75	0.04	0.03	24.07	17.50	0.44	0.32	83.01	60.34	1.51	1.10	F
Sample 120038	0.12	22.45	1.23	0.21	26.16	0.25	0.07	3.71	1.30	0.07	0.02	37.10	12.95	0.68	0.24	176.66	61.69	3.22	1.12	
Sample 120039	0.12	22.45	1.23	0.20	27.11	1.20	0.33	4.65	1.55	0.08	0.03	46.54	15.54	0.85	0.28	232.70	77.71	4.24	1.41	M
Sample 120040	0.13	24.33	1.33	0.17	27.49	2.84	0.78	3.17	2.11	0.06	0.04	31.67	21.12	0.58	0.38	186.32	124.23	3.39	2.26	
Sample 120041	0.12	22.45	1.23	0.32	26.63	2.36	0.63	4.17	1.86	0.08	0.03	41.74	18.57	0.76	0.34	130.44	58.03	2.37	1.06	F

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	Cotton Wool Mass (g)	Mn^{2+} Conc CW (ng/mL)	SD	Mucus Mass (g)	Mn^{2+}Conc ng/mL	%RSD	SD	Sample-CW ng/mL	SD	Sample-CW umol/ml	SD umol/ml	Mn^{2+} Sample-CW ng/mL X10	Mn^{2+} SD ng/mL X10	Mn^{2+} Sample-CW umol/L X10	Mn^{2+} SD umol/ml X10	Mn^{2+} Sample-CW ng/mL per g mucus	Mn^{2+} SD ng/mL per g mucus	Mn^{2+} Sample-CW umol/L per g mucus	Mn^{2+} SD umol/L per g mucus	Sex
Sample 120042	0.13	24.33	1.33	0.21	31.80	1.27	0.40	7.48	1.74	0.14	0.03	74.75	17.35	1.36	0.32	355.97	82.63	6.48	1.50	
Sample 120043	0.15	28.07	1.54	0.20	31.70	0.13	0.04	3.63	1.58	0.07	0.03	36.34	15.77	0.66	0.29	181.72	78.85	3.31	1.44	F
Sample 120044	0.14	26.20	1.43	0.18	32.71	2.72	0.89	6.51	2.32	0.12	0.04	65.12	23.24	1.19	0.42	361.76	129.12	6.58	2.35	
Sample 120045	0.12	22.45	1.23	0.16	24.92	1.33	0.33	2.46	1.56	0.04	0.03	24.63	15.60	0.45	0.28	153.93	97.47	2.80	1.77	F
Sample 120046	0.14	26.20	1.43	0.10	33.24	0.71	0.23	7.04	1.67	0.13	0.03	70.43	16.68	1.28	0.30	704.31	166.85	12.82	3.04	
Sample 120047	0.12	22.45	1.23	0.20	27.01	1.18	0.32	4.56	1.55	0.08	0.03	45.57	15.47	0.83	0.28	227.87	77.34	4.15	1.41	M
Sample 120048	0.13	24.33	1.33	0.12	25.72	0.20	0.05	1.39	1.38	0.03	0.03	13.89	13.82	0.25	0.25	115.76	115.15	2.11	2.10	
Sample 120049	0.11	20.58	1.13	0.22	22.12	2.10	0.46	1.53	1.59	0.03	0.03	15.32	15.91	0.28	0.29	69.63	72.30	1.27	1.32	F
Sample 120050	0.13	24.33	1.33	0.40	25.01	0.96	0.24	0.69	1.57	0.01	0.03	6.85	15.72	0.12	0.29	17.13	39.30	0.31	0.72	
Sample 120051	0.11	20.58	1.13	0.15	21.17	0.83	0.18	0.59	1.30	0.01	0.02	5.87	13.03	0.11	0.24	39.16	86.87	0.71	1.58	M
Sample 120052	0.12	22.45	1.23	0.15	23.10	3.80	0.88	0.64	2.11	0.01	0.04	6.42	21.08	0.12	0.38	42.77	140.51	0.78	2.56	
Sample 120053	0.14	26.20	1.43	0.14	26.84	1.28	0.34	0.64	1.78	0.01	0.03	6.44	17.77	0.12	0.32	45.99	126.94	0.84	2.31	M
Sample 120054	0.13	24.33	1.33	0.36	26.55	0.98	0.26	2.23	1.59	0.04	0.03	22.26	15.91	0.41	0.29	61.82	44.18	1.13	0.80	
Sample 120055	0.12	22.45	1.23	0.40	24.18	1.07	0.26	1.73	1.49	0.03	0.03	17.30	14.89	0.31	0.27	43.25	37.22	0.79	0.68	F
Sample 120056	0.13	24.33	1.33	0.34	26.28	0.41	0.11	1.95	1.44	0.04	0.03	19.50	14.40	0.35	0.26	57.36	42.37	1.04	0.77	
Sample 120057	0.12	22.45	1.23	0.37	24.00	0.16	0.04	1.55	1.27	0.03	0.02	15.46	12.69	0.28	0.23	41.78	34.28	0.76	0.62	M
Sample 120058	0.13	24.33	1.33	0.52	24.50	1.01	0.25	0.18	1.58	0.00	0.03	1.77	15.79	0.03	0.29	3.40	30.37	0.06	0.55	
Sample 120059	0.13	24.33	1.33	0.43	26.28	1.32	0.35	1.95	1.68	0.04	0.03	19.54	16.80	0.36	0.31	45.44	39.06	0.83	0.71	M
Sample 120060	0.13	24.33	1.33	0.47	29.30	2.12	0.62	4.98	1.95	0.09	0.04	49.77	19.52	0.91	0.36	105.90	41.53	1.93	0.76	
Sample 120061	0.12	22.45	1.23	0.31	27.51	0.14	0.04	5.05	1.27	0.09	0.02	50.51	12.68	0.92	0.23	162.94	40.89	2.97	0.74	F
Sample 120062	0.13	24.33	1.33	0.32	28.41	3.17	0.90	4.09	2.23	0.07	0.04	40.88	22.31	0.74	0.41	127.76	69.72	2.33	1.27	
Sample 120063	0.11	20.58	1.13	0.27	26.66	1.97	0.53	6.08	1.65	0.11	0.03	60.77	16.53	1.11	0.30	225.09	61.23	4.10	1.11	M
Sample 120064	0.13	24.33	1.33	0.22	24.73	0.32	0.08	0.40	1.41	0.01	0.03	4.04	14.11	0.07	0.26	18.38	64.11	0.33	1.17	
Sample 120065	0.12	22.45	1.23	0.30	24.92	1.53	0.38	2.47	1.61	0.04	0.03	24.67	16.09	0.45	0.29	82.24	53.64	1.50	0.98	M
Sample 120066	0.13	24.33	1.33	0.35	26.29	0.43	0.11	1.97	1.44	0.04	0.03	19.66	14.43	0.36	0.26	56.18	41.24	1.02	0.75	
Sample 120067	0.11	20.58	1.13	0.17	25.81	0.67	0.17	5.23	1.30	0.10	0.02	52.31	12.99	0.95	0.24	307.72	76.41	5.60	1.39	M
Sample 120068	0.13	24.33	1.33	0.16	25.54	0.89	0.23	1.21	1.56	0.02	0.03	12.14	15.58	0.22	0.28	75.88	97.36	1.38	1.77	

Mn^{2+} Page 3

	Cotton Wool Mass (g)	Mn^{2+} Conc CW (ng/mL)	SD	Mucus Mass (g)	Mn^{2+}Conc ng/mL	%RSD	SD	Sample-CW ng/mL	SD	Sample-CW umol/ml	SD umol/ml	Mn^{2+} Sample-CW ng/mL X10	Mn^{2+} SD ng/mL X10	Mn^{2+} Sample-CW umol/L X10	Mn^{2+} SD umol/ml X10	Mn^{2+} Sample-CW ng/mL per g mucus	Mn^{2+} SD ng/mL per g mucus	Mn^{2+} Sample-CW umol/L per g mucus	Mn^{2+} SD umol/L per g mucus	Sex
Sample 120069	0.14	26.20	1.43	0.54	27.69	2.86	0.79	1.49	2.23	0.03	0.04	14.94	22.26	0.27	0.41	27.66	41.22	0.50	0.75	F
Sample 120070	0.13	24.33	1.33	0.60	23.08	0.38	0.09	-1.24	1.42	-0.02	0.03	-12.44	14.19	-0.23	0.26	-20.73	23.65	-0.38	0.43	
Sample 120071	0.12	22.45	1.23	0.15	27.48	0.24	0.07	5.02	1.29	0.09	0.02	50.22	12.94	0.91	0.24	334.82	86.28	6.09	1.57	F
Sample 120072	0.11	20.58	1.13	0.14	23.61	3.60	0.85	3.02	1.98	0.06	0.04	30.22	19.77	0.55	0.36	215.86	141.22	3.93	2.57	
Sample 120073	0.13	24.33	1.33	0.14	30.99	0.84	0.26	6.67	1.59	0.12	0.03	66.69	15.91	1.21	0.29	476.34	113.67	8.67	2.07	M
Sample 120074	0.13	24.33	1.33	0.19	26.83	2.77	0.74	2.51	2.07	0.05	0.04	25.05	20.74	0.46	0.38	131.85	109.16	2.40	1.99	
Sample 120075	0.13	24.33	1.33	0.32	24.24	1.52	0.37	-0.08	1.70	0.00	0.03	-0.84	16.99	-0.02	0.31	-2.61	53.11	-0.05	0.97	F
Sample 120076	0.13	24.33	1.33	0.36	23.91	0.85	0.20	-0.42	1.53	-0.01	0.03	-4.21	15.34	-0.08	0.28	-11.69	42.60	-0.21	0.78	
Sample 120077	0.11	20.58	1.13	0.43	25.27	1.00	0.25	4.69	1.38	0.09	0.03	46.91	13.79	0.85	0.25	109.09	32.06	1.99	0.58	F
Sample 120078	0.12	22.45	1.23	0.47	26.94	1.84	0.50	4.49	1.73	0.08	0.03	44.86	17.26	0.82	0.31	95.45	36.71	1.74	0.67	
Sample 120079	0.12	22.45	1.23	0.57	26.13	2.53	0.66	3.68	1.89	0.07	0.03	36.77	18.91	0.67	0.34	64.51	33.17	1.17	0.60	M
Sample 120080	0.12	22.45	1.23	0.61	21.82	2.40	0.52	-0.63	1.75	-0.01	0.03	-6.30	17.53	-0.11	0.32	-10.33	28.74	-0.19	0.52	
Sample 120081	0.12	22.45	1.23	0.58	21.57	2.38	0.51	-0.89	1.74	-0.02	0.03	-8.89	17.42	-0.16	0.32	-15.32	30.03	-0.28	0.55	M
Sample 120082	0.12	22.45	1.23	1.07	24.24	2.02	0.49	1.78	1.72	0.03	0.03	17.80	17.18	0.32	0.31	16.64	16.06	0.30	0.29	
Sample 120083	0.12	22.45	1.23	0.51	24.71	0.12	0.03	2.26	1.26	0.04	0.02	22.59	12.59	0.41	0.23	44.30	24.69	0.81	0.45	M
Sample 120084	0.12	22.45	1.23	0.45	25.46	0.68	0.17	3.01	1.40	0.05	0.03	30.08	14.01	0.55	0.26	66.85	31.13	1.22	0.57	
Sample 120085	0.12	22.45	1.23	0.20	22.30	1.05	0.23	-0.15	1.46	0.00	0.03	-1.52	14.62	-0.03	0.27	-7.61	73.11	-0.14	1.33	F
Sample 120086	0.13	24.33	1.33	0.18	25.77	1.38	0.36	1.44	1.69	0.03	0.03	14.41	16.88	0.26	0.31	80.08	93.77	1.46	1.71	
Sample 120087	0.11	20.58	1.13	0.29	24.30	0.61	0.15	3.72	1.28	0.07	0.02	37.17	12.76	0.68	0.23	128.17	44.00	2.33	0.80	M
Sample 120088	0.13	24.33	1.33	0.33	24.40	0.81	0.20	0.07	1.53	0.00	0.03	0.73	15.30	0.01	0.28	2.21	46.35	0.04	0.84	
Sample 120089	0.13	24.33	1.33	0.41	21.96	1.50	0.33	-2.37	1.66	-0.04	0.03	-23.66	16.60	-0.43	0.30	-57.72	40.48	-1.05	0.74	M
Sample 120090	0.12	22.45	1.23	0.41	22.70	3.17	0.72	0.24	1.95	0.00	0.04	2.41	19.49	0.04	0.35	5.88	47.53	0.11	0.87	
Sample 120091	0.12	22.45	1.23	0.32	21.50	0.55	0.12	-0.95	1.35	-0.02	0.02	-9.53	13.48	-0.17	0.25	-29.78	42.14	-0.54	0.77	F
Sample 120092	0.13	24.33	1.33	0.43	20.88	1.65	0.34	-3.45	1.68	-0.06	0.03	-34.47	16.75	-0.63	0.30	-80.17	38.96	-1.46	0.71	
Sample 120093	0.12	22.45	1.23	0.17	21.46	0.43	0.09	-1.00	1.32	-0.02	0.02	-9.97	13.21	-0.18	0.24	-58.64	77.70	-1.07	1.41	F
Sample 120094	0.12	22.45	1.23	0.30	21.92	0.15	0.03	-0.54	1.26	-0.01	0.02	-5.36	12.62	-0.10	0.23	-17.88	42.06	-0.33	0.77	

(e) Zn^{2+}

	Cotton Wool Mass (g)	Zn^{2+} Conc CW (ng/mL)	SD	Mucus Mass (g)	Zn^{2+} Conc ng/mL	%RSD	SD	Sample-CW ng/mL	SD	Sample-CW umol/ml	SD umol/ml	Zn^{2+} Sample-CW ng/mL X10	Zn^{2+} SD ng/mL X10	Zn^{2+} Sample-CW umol/L X10	Zn^{2+} SD umol/ml X10	Zn^{2+} Sample-CW ng/mL per g mucus	Zn^{2+} SD ng/mL per g mucus	Zn^{2+} Sample-CW umol/L per g mucus	Zn^{2+} SD umol/L per g mucus	Sex
Sample 120015	0.15	42.88	10.67	0.38	62.47	0.48	0.30	19.58	10.97	0.30	0.17	195.84	109.68	3.00	1.68	515.37	288.62	7.88	4.41	F
Sample 120016	0.10	28.59	7.11	0.38	127.04	0.70	0.89	98.45	8.00	1.51	0.12	984.48	80.00	15.06	1.22	2590.72	210.53	39.63	3.22	
Sample 120017	0.13	37.17	9.25	0.74	264.16	1.43	3.77	226.99	13.02	3.47	0.20	2269.93	130.17	34.72	1.99	3067.47	175.91	46.92	2.69	F
Sample 120018	0.10	28.59	7.11	0.75	83.54	3.40	2.84	54.95	9.95	0.84	0.15	549.55	99.55	8.41	1.52	732.73	132.73	11.21	2.03	
Sample 120019	0.12	34.31	8.54	0.39	91.79	2.11	1.94	57.48	10.48	0.88	0.16	574.84	104.75	8.79	1.60	1473.94	268.59	22.54	4.11	M
Sample 120020	0.22	62.90	15.65	0.33	1430.58	0.78	11.21	1367.68	26.86	20.92	0.41	13676.84	268.63	209.19	4.11	41444.98	814.03	633.91	12.45	
Sample 120021	0.13	37.17	9.25	0.39	53.05	0.70	0.37	15.88	9.62	0.24	0.15	158.83	96.20	2.43	1.47	407.27	246.66	6.23	3.77	M
Sample 120022	0.13	37.17	9.25	0.46	85.37	1.24	1.06	48.21	10.31	0.74	0.16	482.07	103.08	7.37	1.58	1047.98	224.08	16.03	3.43	
Sample 120023	0.11	31.45	7.82	0.33	87.08	0.66	0.58	55.64	8.40	0.85	0.13	556.36	84.04	8.51	1.29	1685.93	254.66	25.79	3.89	F
Sample 120024	0.14	40.03	9.96	0.33	65.12	0.04	0.02	25.10	9.98	0.38	0.15	250.98	99.83	3.84	1.53	760.54	302.51	11.63	4.63	
Sample 120025	0.11	31.45	7.82	0.23	78.74	0.63	0.50	47.29	8.32	0.72	0.13	472.91	83.23	7.23	1.27	2056.14	361.87	31.45	5.53	M
Sample 120026	0.11	31.45	7.82	0.31	99.92	4.18	4.17	68.47	12.00	1.05	0.18	684.73	119.99	10.47	1.84	2208.82	387.05	33.78	5.92	
Sample 120027	0.12	34.31	8.54	0.62	178.20	0.90	1.61	143.89	10.14	2.20	0.16	1438.92	101.44	22.01	1.55	2320.84	163.62	35.50	2.50	M
Sample 120028	0.13	37.17	9.25	0.51	125.01	0.42	0.52	87.85	9.77	1.34	0.15	878.48	97.67	13.44	1.49	1722.50	191.51	26.35	2.93	
Sample 120029	0.12	34.31	8.54	0.39	126.60	2.43	3.08	92.29	11.61	1.41	0.18	922.94	116.13	14.12	1.78	2366.51	297.76	36.20	4.55	F
Sample 120030	0.12	34.31	8.54	0.32	90.05	1.65	1.48	55.75	10.02	0.85	0.15	557.46	100.20	8.53	1.53	1742.06	313.13	26.65	4.79	
Sample 120031	0.12	34.31	8.54	0.18	103.67	0.20	0.21	69.36	8.75	1.06	0.13	693.63	87.47	10.61	1.34	3853.48	485.93	58.94	7.43	M
Sample 120032	0.13	37.17	9.25	0.20	113.29	1.14	1.29	76.12	10.53	1.16	0.16	761.23	105.35	11.64	1.61	3806.14	526.73	58.22	8.06	
Sample 120033	0.14	40.03	9.96	0.27	65.17	1.81	1.18	25.15	11.14	0.38	0.17	251.46	111.40	3.85	1.70	931.34	412.61	14.25	6.31	F
Sample 120034	0.12	34.31	8.54	0.22	476.99	0.17	0.81	442.68	9.34	6.77	0.14	4426.78	93.42	67.71	1.43	20121.72	424.65	307.77	6.50	
Sample 120035	0.13	37.17	9.25	0.50	98.05	1.28	1.25	60.88	10.50	0.93	0.16	608.82	104.98	9.31	1.61	1217.64	209.96	18.62	3.21	F
Sample 120036	0.12	34.31	8.54	0.37	100.76	1.65	1.66	66.45	10.20	1.02	0.16	664.54	101.96	10.16	1.56	1796.05	275.57	27.47	4.21	
Sample 120037	0.13	37.17	9.25	0.29	70.43	1.79	1.26	33.27	10.51	0.51	0.16	332.66	105.06	5.09	1.61	1147.11	362.26	17.55	5.54	F
Sample 120038	0.12	34.31	8.54	0.21	61.97	3.34	2.07	27.66	10.61	0.42	0.16	276.58	106.05	4.23	1.62	1317.07	505.02	20.14	7.72	
Sample 120039	0.12	34.31	8.54	0.20	61.86	1.26	0.78	27.55	9.32	0.42	0.14	275.55	93.18	4.21	1.43	1377.75	465.92	21.07	7.13	M
Sample 120040	0.13	37.17	9.25	0.17	87.02	2.77	2.41	49.85	11.66	0.76	0.18	498.50	116.60	7.62	1.78	2932.36	685.88	44.85	10.49	
Sample 120041	0.12	34.31	8.54	0.32	106.92	1.84	1.96	72.61	10.50	1.11	0.16	726.14	105.01	11.11	1.61	2269.18	328.14	34.71	5.02	F

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	Cotton Wool Mass (g)	Zn^{2+} Conc CW (ng/mL)	SD	Mucus Mass (g)	Zn^{2+} Conc ng/mL	%RSD	SD	Sample-CW ng/mL	SD	Sample-CW umol/ml	SD umol/ml	Zn^{2+} Sample-CW ng/mL X10	Zn^{2+} SD ng/mL X10	Zn^{2+} Sample-CW umol/L X10	Zn^{2+} SD umol/ml X10	Zn^{2+} Sample-CW ng/mL per g mucus	Zn^{2+} SD ng/mL per g mucus	Zn^{2+} Sample-CW umol/L per g mucus	Zn^{2+} SD umol/L per g mucus	Sex
Sample 120042	0.13	37.17	9.25	0.21	103.44	2.87	2.97	66.27	12.22	1.01	0.19	662.74	122.19	10.14	1.87	3155.93	581.85	48.27	8.90	
Sample 120043	0.15	42.88	10.67	0.20	71.83	3.85	2.77	28.95	13.44	0.44	0.21	289.48	134.39	4.43	2.06	1447.40	671.95	22.14	10.28	F
Sample 120044	0.14	40.03	9.96	0.18	68.26	1.20	0.82	28.23	10.78	0.43	0.16	282.31	107.77	4.32	1.65	1568.37	598.73	23.99	9.16	
Sample 120045	0.12	34.31	8.54	0.16	103.00	0.90	0.92	68.69	9.46	1.05	0.14	686.89	94.61	10.51	1.45	4293.05	591.28	65.66	9.04	F
Sample 120046	0.14	40.03	9.96	0.10	62.87	2.16	1.36	22.84	11.32	0.35	0.17	228.43	113.17	3.49	1.73	2284.32	1131.72	34.94	17.31	
Sample 120047	0.12	34.31	8.54	0.20	111.18	2.37	2.64	76.87	11.18	1.18	0.17	768.68	111.75	11.76	1.71	3843.40	558.76	58.79	8.55	M
Sample 120048	0.13	37.17	9.25	0.12	100.80	1.26	1.27	63.64	10.52	0.97	0.16	636.37	105.16	9.73	1.61	5303.04	876.29	81.11	13.40	
Sample 120049	0.11	31.45	7.82	0.22	84.26	1.20	1.01	52.81	8.84	0.81	0.14	528.13	88.39	8.08	1.35	2400.60	401.79	36.72	6.15	F
Sample 120050	0.13	37.17	9.25	0.40	99.24	0.79	0.78	62.07	10.03	0.95	0.15	620.75	100.30	9.49	1.53	1551.87	250.76	23.74	3.84	
Sample 120051	0.11	31.45	7.82	0.15	92.81	1.86	1.73	61.36	9.55	0.94	0.15	613.62	95.55	9.39	1.46	4090.83	636.99	62.57	9.74	M
Sample 120052	0.12	34.31	8.54	0.15	105.94	3.34	3.54	71.63	12.08	1.10	0.18	716.31	120.78	10.96	1.85	4775.39	805.17	73.04	12.32	
Sample 120053	0.14	40.03	9.96	0.14	76.84	1.03	0.79	36.82	10.75	0.56	0.16	368.17	107.49	5.63	1.64	2629.77	767.76	40.22	11.74	M
Sample 120054	0.13	37.17	9.25	0.36	90.96	3.60	3.27	53.79	12.52	0.82	0.19	537.94	125.18	8.23	1.91	1494.28	347.73	22.86	5.32	
Sample 120055	0.12	34.31	8.54	0.40	72.98	0.23	0.17	38.67	8.71	0.59	0.13	386.71	87.07	5.91	1.33	966.79	217.68	14.79	3.33	F
Sample 120056	0.13	37.17	9.25	0.34	86.88	0.14	0.13	49.71	9.37	0.76	0.14	497.09	93.73	7.60	1.43	1462.03	275.68	22.36	4.22	
Sample 120057	0.12	34.31	8.54	0.37	92.55	0.56	0.52	58.24	9.05	0.89	0.14	582.43	90.52	8.91	1.38	1574.13	244.64	24.08	3.74	M
Sample 120058	0.13	37.17	9.25	0.52	123.62	0.94	1.17	86.45	10.42	1.32	0.16	864.50	104.15	13.22	1.59	1662.49	200.29	25.43	3.06	
Sample 120059	0.13	37.17	9.25	0.43	77.06	4.69	3.61	39.90	12.86	0.61	0.20	398.98	128.60	6.10	1.97	927.85	299.07	14.19	4.57	M
Sample 120060	0.13	37.17	9.25	0.47	162.20	2.40	3.90	125.03	13.15	1.91	0.20	1250.31	131.45	19.12	2.01	2660.23	279.69	40.69	4.28	
Sample 120061	0.12	34.31	8.54	0.31	92.29	1.67	1.54	57.99	10.08	0.89	0.15	579.86	100.78	8.87	1.54	1870.51	325.10	28.61	4.97	F
Sample 120062	0.13	37.17	9.25	0.32	114.96	1.84	2.11	77.80	11.36	1.19	0.17	777.96	113.59	11.90	1.74	2431.14	354.96	37.18	5.43	
Sample 120063	0.11	31.45	7.82	0.27	88.31	0.22	0.20	56.86	8.02	0.87	0.12	568.63	80.22	8.70	1.23	2106.05	297.10	32.21	4.54	M
Sample 120064	0.13	37.17	9.25	0.22	51.63	0.34	0.18	14.46	9.42	0.22	0.14	144.61	94.24	2.21	1.44	657.32	428.37	10.05	6.55	
Sample 120065	0.12	34.31	8.54	0.30	87.62	2.56	2.25	53.31	10.78	0.82	0.16	533.15	107.83	8.15	1.65	1777.16	359.43	27.18	5.50	M
Sample 120066	0.13	37.17	9.25	0.35	106.43	0.16	0.17	69.26	9.41	1.06	0.14	692.61	94.14	10.59	1.44	1978.90	268.98	30.27	4.11	
Sample 120067	0.11	31.45	7.82	0.17	81.81	1.12	0.92	50.36	8.74	0.77	0.13	503.57	87.40	7.70	1.34	2962.18	514.09	45.31	7.86	M
Sample 120068	0.13	37.17	9.25	0.16	81.87	0.15	0.13	44.71	9.37	0.68	0.14	447.06	93.73	6.84	1.43	2794.11	585.81	42.74	8.96	

Zn^{2+} Page 3

	Cotton Wool Mass (g)	Zn^{2+} Conc CW (ng/mL)	SD	Mucus Mass (g)	Zn^{2+} Conc ng/mL	%RSD	SD	Sample-CW ng/mL	SD	Sample-CW umol/ml	SD umol/ml	Zn^{2+} Sample-CW ng/mL X10	Zn^{2+} SD ng/mL X10	Zn^{2+} Sample-CW umol/L X10	Zn^{2+} SD umol/ml X10	Zn^{2+} Sample-CW ng/mL per g mucus	Zn^{2+} SD ng/mL per g mucus	Zn^{2+} Sample-CW umol/L per g mucus	Zn^{2+} SD umol/L per g mucus	Sex
Sample 120069	0.14	40.03	9.96	0.54	68.61	2.93	2.01	28.59	11.97	0.44	0.18	285.89	119.72	4.37	1.83	529.43	221.69	8.10	3.39	F
Sample 120070	0.13	37.17	9.25	0.60	82.94	2.62	2.18	45.77	11.42	0.70	0.17	457.69	114.22	7.00	1.75	762.81	190.37	11.67	2.91	
Sample 120071	0.12	34.31	8.54	0.15	63.00	2.27	1.43	28.70	9.97	0.44	0.15	286.96	99.66	4.39	1.52	1913.08	664.41	29.26	10.16	F
Sample 120072	0.11	31.45	7.82	0.14	62.59	1.13	0.70	31.14	8.53	0.48	0.13	311.39	85.29	4.76	1.30	2224.24	609.19	34.02	9.32	
Sample 120073	0.13	37.17	9.25	0.14	73.31	0.02	0.01	36.14	9.26	0.55	0.14	361.42	92.60	5.53	1.42	2581.55	661.46	39.49	10.12	M
Sample 120074	0.13	37.17	9.25	0.19	70.00	0.89	0.62	32.84	9.87	0.50	0.15	328.36	98.69	5.02	1.51	1728.19	519.42	26.43	7.94	
Sample 120075	0.13	37.17	9.25	0.32	60.58	0.48	0.29	23.41	9.54	0.36	0.15	234.11	95.38	3.58	1.46	731.61	298.05	11.19	4.56	F
Sample 120076	0.13	37.17	9.25	0.36	86.17	1.42	1.22	49.01	10.47	0.75	0.16	490.07	104.72	7.50	1.60	1361.31	290.88	20.82	4.45	
Sample 120077	0.11	31.45	7.82	0.43	64.77	0.35	0.23	33.32	8.05	0.51	0.12	333.24	80.51	5.10	1.23	774.97	187.23	11.85	2.86	F
Sample 120078	0.12	34.31	8.54	0.47	62.47	0.58	0.36	28.16	8.90	0.43	0.14	281.63	89.00	4.31	1.36	599.22	189.35	9.17	2.90	
Sample 120079	0.12	34.31	8.54	0.57	54.51	4.24	2.31	20.21	10.85	0.31	0.17	202.06	108.48	3.09	1.66	354.50	190.32	5.42	2.91	M
Sample 120080	0.12	34.31	8.54	0.61	65.84	0.18	0.12	31.53	8.65	0.48	0.13	315.28	86.55	4.82	1.32	516.85	141.88	7.91	2.17	
Sample 120081	0.12	34.31	8.54	0.58	65.67	0.58	0.38	31.36	8.92	0.48	0.14	313.57	89.18	4.80	1.36	540.64	153.75	8.27	2.35	M
Sample 120082	0.12	34.31	8.54	1.07	97.05	0.63	0.61	62.75	9.14	0.96	0.14	627.45	91.43	9.60	1.40	586.41	85.45	8.97	1.31	
Sample 120083	0.12	34.31	8.54	0.51	60.15	0.64	0.39	25.84	8.92	0.40	0.14	258.38	89.21	3.95	1.36	506.63	174.92	7.75	2.68	M
Sample 120084	0.12	34.31	8.54	0.45	65.13	1.27	0.82	30.83	9.36	0.47	0.14	308.27	93.60	4.72	1.43	685.04	208.01	10.48	3.18	
Sample 120085	0.12	34.31	8.54	0.20	45.09	0.87	0.39	10.78	8.93	0.16	0.14	107.79	89.30	1.65	1.37	538.94	446.49	8.24	6.83	F
Sample 120086	0.13	37.17	9.25	0.18	65.89	0.48	0.32	28.72	9.57	0.44	0.15	287.19	95.67	4.39	1.46	1595.50	531.49	24.40	8.13	
Sample 120087	0.11	31.45	7.82	0.29	72.78	2.10	1.53	41.33	9.35	0.63	0.14	413.33	93.52	6.32	1.43	1425.27	322.50	21.80	4.93	M
Sample 120088	0.13	37.17	9.25	0.33	56.20	2.17	1.22	19.03	10.47	0.29	0.16	190.32	104.67	2.91	1.60	576.72	317.19	8.82	4.85	
Sample 120089	0.13	37.17	9.25	0.41	49.90	1.35	0.67	12.73	9.92	0.19	0.15	127.31	99.20	1.95	1.52	310.51	241.95	4.75	3.70	M
Sample 120090	0.12	34.31	8.54	0.41	67.40	1.65	1.12	33.10	9.65	0.51	0.15	330.96	96.51	5.06	1.48	807.23	235.39	12.35	3.60	
Sample 120091	0.12	34.31	8.54	0.32	50.10	0.40	0.20	15.79	8.73	0.24	0.13	157.87	87.34	2.41	1.34	493.35	272.94	7.55	4.17	F
Sample 120092	0.13	34.31	8.54	0.43	59.02	2.44	1.44	24.71	9.98	0.38	0.15	247.11	99.78	3.78	1.53	574.67	232.06	8.79	3.55	
Sample 120093	0.12	34.31	8.54	0.17	55.29	0.89	0.49	20.98	9.03	0.32	0.14	209.84	90.27	3.21	1.38	1234.37	531.00	18.88	8.12	F
Sample 120094	0.12	34.31	8.54	0.30	59.11	1.55	0.92	24.80	9.45	0.38	0.14	247.97	94.54	3.79	1.45	826.57	315.12	12.64	4.82	

Appendix Q Full AAS Results Tables for All Bulk Metals in the 80 Mucus Samples

Result Tables for (a) Na^+, (b) K^+, (c), Ca^{2+} and (d) Mg^{2+}

Negative values can be seen in a small number of samples once the average calculated mass of the cotton wool pellets were removed. It was not possible to calculate an exact mass for the cations in each individual pellet.

(a) Na^+

	Cotton Wool Mass (g)	CW Na^+ Conc mg/L	CW SD	Mucus Mass (g)	Mucus Na^+ Conc mg/L	Mucus SD	Na^+ conc Mucus - CW mg/L	Na^+ conc SD mg/L	Na^+ conc Mucus - CW mmol/L	Na^+ conc SD mmol/L	Na^+ Conc (mg/L) X1000	Na^+ Conc (mg/L) SD X1000	Na^+ Conc (mmol/L) X1000	Na^+ Conc (mmol/L) SD X1000	Na^+ conc mg/L per g mucus	Na^+ conc mg/L per g mucus SD	Na^+ conc mmol/L per g mucus	Na^+ conc mmol/L per g mucus SD	Sex
Sample 120015	0.15	0.04	0.01	0.38	0.87	0.01	0.83	0.02	0.04	0.00	831.75	16.69	36.18	0.73	2188.83	43.91	95.21	1.91	F
Sample 120016	0.10	0.03	0.00	0.38	1.08	0.01	1.05	0.01	0.05	0.00	1051.57	10.51	45.74	0.46	2767.29	27.65	120.37	1.20	
Sample 120017	0.13	0.03	0.01	0.74	1.49	0.02	1.46	0.03	0.06	0.00	1455.28	27.40	63.30	1.19	1966.59	37.02	85.55	1.61	F
Sample 120018	0.10	0.03	0.00	0.75	1.89	0.05	1.87	0.05	0.08	0.00	1868.84	52.27	81.29	2.27	2491.78	69.69	108.39	3.03	
Sample 120019	0.12	0.03	0.01	0.39	1.11	0.01	1.08	0.02	0.05	0.00	1081.97	19.13	47.06	0.83	2774.27	49.05	120.68	2.13	M
Sample 120020	0.22	0.06	0.01	0.33	1.07	0.02	1.02	0.03	0.04	0.00	1016.43	31.51	44.21	1.37	3080.10	95.48	133.98	4.15	
Sample 120021	0.13	0.03	0.01	0.39	1.29	0.06	1.26	0.06	0.05	0.00	1257.19	64.64	54.69	2.81	3223.56	165.76	140.22	7.21	M
Sample 120022	0.13	0.03	0.01	0.46	1.37	0.01	1.34	0.02	0.06	0.00	1337.47	15.89	58.18	0.69	2907.55	34.54	126.48	1.50	
Sample 120023	0.11	0.03	0.00	0.33	1.11	0.03	1.08	0.03	0.05	0.00	1077.44	33.47	46.87	1.46	3264.96	101.44	142.02	4.41	F
Sample 120024	0.14	0.04	0.01	0.33	1.06	0.01	1.03	0.02	0.04	0.00	1027.64	16.76	44.70	0.73	3114.06	50.78	135.46	2.21	
Sample 120025	0.11	0.03	0.00	0.23	0.53	0.01	0.50	0.02	0.02	0.00	496.95	15.61	21.62	0.68	2160.64	67.88	93.99	2.95	M
Sample 120026	0.11	0.03	0.00	0.31	0.90	0.02	0.88	0.03	0.04	0.00	876.32	26.54	38.12	1.15	2826.82	85.60	122.96	3.72	
Sample 120027	0.12	0.03	0.01	0.62	1.94	0.03	1.90	0.03	0.08	0.00	1904.80	34.01	82.86	1.48	3072.25	54.85	133.64	2.39	M
Sample 120028	0.13	0.03	0.01	0.51	1.65	0.02	1.62	0.02	0.07	0.00	1617.88	24.11	70.38	1.05	3172.31	47.27	137.99	2.06	
Sample 120029	0.12	0.03	0.01	0.39	1.13	0.02	1.09	0.02	0.05	0.00	1094.34	23.08	47.60	1.00	2805.99	59.17	122.06	2.57	F
Sample 120030	0.12	0.03	0.01	0.32	0.99	0.02	0.96	0.03	0.04	0.00	960.63	27.84	41.79	1.21	3001.97	87.01	130.58	3.78	
Sample 120031	0.12	0.03	0.01	0.18	0.63	0.01	0.60	0.01	0.03	0.00	599.28	11.61	26.07	0.50	3329.31	64.49	144.82	2.81	M
Sample 120032	0.13	0.03	0.01	0.20	0.75	0.01	0.71	0.01	0.03	0.00	713.27	14.03	31.03	0.61	3566.36	70.15	155.13	3.05	
Sample 120033	0.14	0.04	0.01	0.27	0.91	0.02	0.88	0.02	0.04	0.00	878.66	23.06	38.22	1.00	3254.28	85.40	141.56	3.71	F
Sample 120034	0.12	0.03	0.01	0.22	0.76	0.00	0.73	0.01	0.03	0.00	733.19	8.26	31.89	0.36	3332.69	37.53	144.97	1.63	
Sample 120035	0.13	0.03	0.01	0.50	1.48	0.02	1.44	0.03	0.06	0.00	1442.00	29.93	62.73	1.30	2883.99	59.86	125.45	2.60	F
Sample 120036	0.12	0.03	0.01	0.37	1.16	0.01	1.13	0.02	0.05	0.00	1128.62	18.92	49.09	0.82	3050.34	51.13	132.69	2.22	
Sample 120037	0.13	0.03	0.01	0.29	0.91	0.01	0.88	0.02	0.04	0.00	879.32	17.91	38.25	0.78	3032.12	61.76	131.89	2.69	F
Sample 120038	0.12	0.03	0.01	0.21	0.68	0.00	0.65	0.01	0.03	0.00	650.09	9.35	28.28	0.41	3095.65	44.54	134.66	1.94	
Sample 120039	0.12	0.03	0.01	0.20	0.57	0.00	0.54	0.01	0.02	0.00	536.59	9.36	23.34	0.41	2682.97	46.79	116.71	2.04	M
Sample 120040	0.13	0.03	0.01	0.17	0.60	0.02	0.57	0.03	0.02	0.00	565.12	25.10	24.58	1.09	3324.23	147.65	144.60	6.42	
Sample 120041	0.12	0.03	0.01	0.32	0.78	0.01	0.75	0.01	0.03	0.00	747.68	13.67	32.52	0.59	2336.49	42.73	101.64	1.86	F

Na+ page 2

	Cotton Wool Mass (g)	CW Na^+ Conc mg/L	CW SD	Mucus Mass (g)	Mucus Na^+ Conc mg/L	Mucus SD	Na^+ conc Mucus - CW mg/L	Na^+ conc SD mg/L	Na^+ conc Mucus - CW mmol/L	Na^+ conc SD mmol/L	Na^+ Conc (mg/L) X1000	Na^+ Conc (mg/L) SD X1000	Na^+ Conc (mmol/L) X1000	Na^+ Conc (mmol/L) SD X1000	Na^+ conc mg/L per g mucus	Na^+ conc mg/L per g mucus SD	Na^+ conc mmol/L per g mucus	Na^+ conc mmol/L per g mucus SD	Sex
Sample 120042	0.13	0.03	0.01	0.21	0.85	0.00	0.81	0.01	0.04	0.00	813.02	9.63	35.37	0.42	3871.53	45.84	168.41	1.99	
Sample 120043	0.15	0.04	0.01	0.20	0.69	0.01	0.65	0.02	0.03	0.00	652.43	17.39	28.38	0.76	3262.13	86.97	141.90	3.78	F
Sample 120044	0.14	0.04	0.01	0.18	0.62	0.01	0.59	0.01	0.03	0.00	588.04	13.61	25.58	0.59	3266.88	75.62	142.11	3.29	
Sample 120045	0.12	0.03	0.01	0.16	0.59	0.01	0.56	0.02	0.02	0.00	562.79	15.02	24.48	0.65	3517.43	93.90	153.00	4.08	F
Sample 120046	0.14	0.04	0.01	0.10	0.34	0.00	0.30	0.01	0.01	0.00	302.57	8.37	13.16	0.36	3025.67	83.66	131.61	3.64	
Sample 120047	0.12	0.03	0.01	0.20	0.74	0.00	0.71	0.01	0.03	0.00	705.21	6.35	30.68	0.28	3526.06	31.77	153.38	1.38	M
Sample 120048	0.13	0.03	0.01	0.12	0.46	0.01	0.43	0.01	0.02	0.00	431.29	13.96	18.76	0.61	3594.06	116.30	156.34	5.06	
Sample 120049	0.11	0.03	0.00	0.22	0.71	0.01	0.68	0.02	0.03	0.00	681.34	19.50	29.64	0.85	3096.99	88.65	134.72	3.86	F
Sample 120050	0.13	0.03	0.01	0.40	1.30	0.02	1.27	0.03	0.06	0.00	1271.22	26.83	55.30	1.17	3178.05	67.07	138.24	2.92	
Sample 120051	0.11	0.03	0.00	0.15	0.51	0.00	0.48	0.01	0.02	0.00	480.05	6.75	20.88	0.29	3200.34	45.00	139.21	1.96	M
Sample 120052	0.12	0.03	0.01	0.15	0.60	0.01	0.57	0.01	0.02	0.00	568.77	11.17	24.74	0.49	3791.77	74.48	164.94	3.24	
Sample 120053	0.14	0.04	0.01	0.14	0.44	0.00	0.41	0.01	0.02	0.00	408.54	8.76	17.77	0.38	2918.17	62.57	126.94	2.72	M
Sample 120054	0.13	0.03	0.01	0.36	1.07	0.01	1.03	0.02	0.05	0.00	1034.77	16.44	45.01	0.72	2874.37	45.66	125.03	1.99	
Sample 120055	0.12	0.03	0.01	0.40	1.11	0.03	1.08	0.03	0.05	0.00	1078.31	31.38	46.91	1.37	2695.78	78.45	117.26	3.41	F
Sample 120056	0.13	0.03	0.01	0.34	1.12	0.02	1.09	0.02	0.05	0.00	1086.33	21.56	47.25	0.94	3195.09	63.42	138.98	2.76	
Sample 120057	0.12	0.03	0.01	0.37	1.21	0.01	1.18	0.02	0.05	0.00	1184.17	20.05	51.51	0.87	3200.45	54.19	139.22	2.36	M
Sample 120058	0.13	0.03	0.01	0.52	1.70	0.03	1.67	0.04	0.07	0.00	1669.14	36.07	72.61	1.57	3209.89	69.36	139.63	3.02	
Sample 120059	0.13	0.03	0.01	0.43	1.50	0.03	1.47	0.04	0.06	0.00	1466.61	40.56	63.80	1.76	3410.73	94.32	148.36	4.10	M
Sample 120060	0.13	0.03	0.01	0.47	1.78	0.02	1.75	0.03	0.08	0.00	1748.26	29.06	76.05	1.26	3719.71	61.83	161.80	2.69	
Sample 120061	0.12	0.03	0.01	0.31	0.97	0.02	0.94	0.02	0.04	0.00	938.55	24.53	40.83	1.07	3027.57	79.12	131.70	3.44	F
Sample 120062	0.13	0.03	0.01	0.32	1.02	0.01	0.99	0.01	0.04	0.00	987.33	14.99	42.95	0.65	3085.40	46.83	134.21	2.04	
Sample 120063	0.11	0.03	0.00	0.27	0.79	0.01	0.76	0.02	0.03	0.00	762.12	17.47	33.15	0.76	2822.66	64.70	122.78	2.81	M
Sample 120064	0.13	0.03	0.01	0.22	0.77	0.02	0.73	0.03	0.03	0.00	734.19	25.43	31.94	1.11	3337.24	115.60	145.17	5.03	
Sample 120065	0.12	0.03	0.01	0.30	0.96	0.00	0.93	0.01	0.04	0.00	933.94	7.82	40.63	0.34	3113.13	26.07	135.42	1.13	M
Sample 120066	0.13	0.03	0.01	0.35	1.07	0.01	1.04	0.01	0.05	0.00	1040.96	12.68	45.28	0.55	2974.17	36.22	129.37	1.58	
Sample 120067	0.11	0.03	0.00	0.17	0.70	0.01	0.67	0.02	0.03	0.00	673.53	18.61	29.30	0.81	3961.97	109.47	172.34	4.76	M
Sample 120068	0.13	0.03	0.01	0.16	0.48	0.00	0.45	0.01	0.02	0.00	450.34	10.88	19.59	0.47	2814.63	68.00	122.43	2.96	

	Cotton Wool Mass (g)	CW Na^+ Conc mg/L	CW SD	Mucus Mass (g)	Mucus Na^+ Conc mg/L	Mucus SD	Na^+ conc Mucus - CW mg/L	Na^+ conc SD mg/L	Na^+ conc Mucus - CW mmol/L	Na^+ conc SD mmol/L	Na^+ Conc (mg/L) X1000	Na^+ Conc (mg/L) SD X1000	Na^+ Conc (mmol/L) X1000	Na^+ Conc (mmol/L) SD X1000	Na^+ conc mg/L per g mucus	Na^+ conc mg/L per g mucus SD	Na^+ conc mmol/L per g mucus	Na^+ conc mmol/L per g mucus SD	Sex
Sample 120069	0.14	0.04	0.01	0.54	1.56	0.02	1.53	0.03	0.07	0.00	1526.02	30.38	66.38	1.32	2825.96	56.26	122.93	2.45	F
Sample 120070	0.13	0.03	0.01	0.60	0.83	0.02	0.79	0.02	0.03	0.00	793.68	21.22	34.52	0.92	1322.80	35.36	57.54	1.54	
Sample 120071	0.12	0.03	0.01	0.15	0.42	0.00	0.39	0.01	0.02	0.00	386.78	8.78	16.82	0.38	2578.54	58.53	112.16	2.55	F
Sample 120072	0.11	0.03	0.00	0.14	0.39	0.01	0.36	0.01	0.02	0.00	358.55	13.77	15.60	0.60	2561.06	98.33	111.40	4.28	
Sample 120073	0.13	0.03	0.01	0.14	0.45	0.00	0.41	0.01	0.02	0.00	413.40	9.53	17.98	0.41	2952.83	68.09	128.45	2.96	M
Sample 120074	0.13	0.03	0.01	0.19	0.66	0.00	0.63	0.01	0.03	0.00	625.56	10.60	27.21	0.46	3292.41	55.81	143.22	2.43	
Sample 120075	0.13	0.03	0.01	0.32	0.92	0.03	0.89	0.04	0.04	0.00	890.61	37.66	38.74	1.64	2783.14	117.70	121.06	5.12	F
Sample 120076	0.13	0.03	0.01	0.36	0.98	0.03	0.94	0.04	0.04	0.00	944.82	35.78	41.10	1.56	2624.50	99.40	114.16	4.32	
Sample 120077	0.11	0.03	0.00	0.43	1.16	0.01	1.13	0.02	0.05	0.00	1131.28	19.91	49.21	0.87	2630.87	46.30	114.44	2.01	F
Sample 120078	0.12	0.03	0.01	0.47	1.28	0.02	1.25	0.03	0.05	0.00	1246.10	25.71	54.20	1.12	2651.28	54.70	115.33	2.38	
Sample 120079	0.12	0.03	0.01	0.57	1.55	0.02	1.52	0.02	0.07	0.00	1519.66	23.94	66.10	1.04	2666.07	42.00	115.97	1.83	M
Sample 120080	0.12	0.03	0.01	0.61	1.72	0.04	1.69	0.05	0.07	0.00	1685.70	46.43	73.33	2.02	2763.45	76.11	120.21	3.31	
Sample 120081	0.12	0.03	0.01	0.58	1.40	0.03	1.37	0.04	0.06	0.00	1374.16	37.01	59.77	1.61	2369.25	63.81	103.06	2.78	M
Sample 120082	0.12	0.03	0.01	1.07	2.54	0.33	2.51	0.34	0.11	0.01	2506.33	336.36	109.02	14.63	2342.37	314.35	101.89	13.67	
Sample 120083	0.12	0.03	0.01	0.51	1.47	0.01	1.44	0.01	0.06	0.00	1439.79	12.80	62.63	0.56	2823.12	25.09	122.80	1.09	M
Sample 120084	0.12	0.03	0.01	0.45	1.39	0.02	1.36	0.02	0.06	0.00	1362.41	22.10	59.26	0.96	3027.59	49.11	131.70	2.14	
Sample 120085	0.12	0.03	0.01	0.20	0.69	0.03	0.66	0.03	0.03	0.00	660.46	32.28	28.73	1.40	3302.32	161.38	143.65	7.02	F
Sample 120086	0.13	0.03	0.01	0.18	0.55	0.01	0.52	0.02	0.02	0.00	515.55	16.48	22.43	0.72	2864.19	91.53	124.59	3.98	
Sample 120087	0.11	0.03	0.00	0.29	0.80	0.01	0.77	0.02	0.03	0.00	770.79	17.64	33.53	0.77	2657.91	60.83	115.62	2.65	M
Sample 120088	0.13	0.03	0.01	0.33	0.89	0.03	0.86	0.04	0.04	0.00	860.10	40.64	37.41	1.77	2606.35	123.16	113.37	5.36	
Sample 120089	0.13	0.03	0.01	0.41	1.12	0.02	1.09	0.03	0.05	0.00	1085.29	27.35	47.21	1.19	2647.06	66.72	115.14	2.90	M
Sample 120090	0.12	0.03	0.01	0.41	1.40	0.02	1.37	0.03	0.06	0.00	1369.68	25.38	59.58	1.10	3340.68	61.90	145.32	2.69	
Sample 120091	0.12	0.03	0.01	0.32	1.10	0.00	1.07	0.01	0.05	0.00	1069.14	8.96	46.51	0.39	3341.06	27.99	145.33	1.22	F
Sample 120092	0.13	0.03	0.01	0.43	1.40	0.02	1.37	0.03	0.06	0.00	1366.24	26.07	59.43	1.13	3177.30	60.62	138.21	2.64	
Sample 120093	0.12	0.03	0.01	0.17	0.63	0.02	0.60	0.02	0.03	0.00	599.61	21.69	26.08	0.94	3527.11	127.57	153.43	5.55	F
Sample 120094	0.12	0.03	0.01	0.30	0.98	0.01	0.95	0.02	0.04	0.00	945.64	20.00	41.13	0.87	3152.15	66.67	137.12	2.90	

(b) K^+

	Cotton Wool Mass (g)	CW K^+ Conc mg/L	CW SD	Mucus Mass (g)	Mucus K^+ Conc mg/L	Mucus SD	K^+ conc Mucus - CW mg/L	K^+ conc SD mg/L	K^+ conc Mucus - CW mmol/L	K^+ conc SD mmol/L	K^+ Conc (mg/L) X1000	K^+ Conc (mg/L) SD X1000	K^+ Conc (mmol/L) X1000	K^+ Conc (mmol/L) SD X1000	K^+ conc mg/L per g mucus	K^+ conc mg/L per g mucus SD	K^+ conc mmol/L per g mucus	K^+ conc mmol/L per g mucus SD	Sex
Sample 120015	0.15	0.31	0.01	0.38	2.11	0.00	1.80	0.01	0.05	0.00	179.85	1.02	4.60	0.03	473.30	2.70	12.11	0.07	F
Sample 120016	0.10	0.21	0.01	0.38	2.89	0.05	2.68	0.06	0.07	0.00	268.05	5.64	6.86	0.14	705.40	14.83	18.04	0.38	
Sample 120017	0.13	0.27	0.01	0.74	4.15	0.03	3.88	0.04	0.10	0.00	387.57	3.81	9.91	0.10	523.74	5.14	13.40	0.13	F
Sample 120018	0.10	0.21	0.01	0.75	5.72	0.14	5.51	0.14	0.14	0.00	551.13	14.29	14.10	0.37	734.84	19.05	18.79	0.49	
Sample 120019	0.12	0.25	0.01	0.39	2.99	0.01	2.74	0.02	0.07	0.00	273.51	2.05	7.00	0.05	701.31	5.26	17.94	0.13	M
Sample 120020	0.22	0.46	0.01	0.33	3.48	0.04	3.02	0.05	0.08	0.00	301.88	5.19	7.72	0.13	914.78	15.73	23.40	0.40	
Sample 120021	0.13	0.27	0.01	0.39	2.80	0.03	2.52	0.04	0.06	0.00	252.47	3.84	6.46	0.10	647.36	9.84	16.56	0.25	M
Sample 120022	0.13	0.27	0.01	0.46	3.18	0.01	2.90	0.02	0.07	0.00	290.40	2.09	7.43	0.05	631.31	4.54	16.15	0.12	
Sample 120023	0.11	0.23	0.01	0.33	1.91	0.04	1.68	0.04	0.04	0.00	167.83	4.27	4.29	0.11	508.58	12.93	13.01	0.33	F
Sample 120024	0.14	0.29	0.01	0.33	2.37	0.06	2.08	0.06	0.05	0.00	207.84	6.35	5.32	0.16	629.82	19.24	16.11	0.49	
Sample 120025	0.11	0.23	0.01	0.23	1.12	0.01	0.89	0.02	0.02	0.00	88.90	2.05	2.27	0.05	386.52	8.91	9.89	0.23	M
Sample 120026	0.11	0.23	0.01	0.31	1.93	0.05	1.70	0.06	0.04	0.00	169.70	5.62	4.34	0.14	547.43	18.14	14.00	0.46	
Sample 120027	0.12	0.25	0.01	0.62	3.58	0.16	3.33	0.17	0.09	0.00	333.34	16.63	8.53	0.43	537.64	26.82	13.75	0.69	M
Sample 120028	0.13	0.27	0.01	0.51	3.39	0.17	3.12	0.18	0.08	0.00	312.19	18.08	7.98	0.46	612.14	35.44	15.66	0.91	
Sample 120029	0.12	0.25	0.01	0.39	3.23	0.12	2.98	0.12	0.08	0.00	298.29	12.34	7.63	0.32	764.84	31.65	19.56	0.81	F
Sample 120030	0.12	0.25	0.01	0.32	2.51	0.07	2.26	0.08	0.06	0.00	225.83	7.75	5.78	0.20	705.72	24.21	18.05	0.62	
Sample 120031	0.12	0.25	0.01	0.18	1.17	0.04	0.92	0.05	0.02	0.00	91.85	4.58	2.35	0.12	510.27	25.43	13.05	0.65	M
Sample 120032	0.13	0.27	0.01	0.20	1.13	0.03	0.86	0.04	0.02	0.00	85.51	3.88	2.19	0.10	427.53	19.41	10.93	0.50	
Sample 120033	0.14	0.29	0.01	0.27	2.01	0.07	1.72	0.08	0.04	0.00	172.11	8.01	4.40	0.20	637.45	29.65	16.30	0.76	F
Sample 120034	0.12	0.25	0.01	0.22	1.68	0.03	1.43	0.04	0.04	0.00	143.27	4.16	3.66	0.11	651.25	18.89	16.66	0.48	
Sample 120035	0.13	0.27	0.01	0.50	3.43	0.29	3.16	0.29	0.08	0.01	315.74	29.34	8.08	0.75	631.48	58.69	16.15	1.50	F
Sample 120036	0.12	0.25	0.01	0.37	2.86	0.10	2.61	0.11	0.07	0.00	260.63	10.64	6.67	0.27	704.41	28.75	18.02	0.74	
Sample 120037	0.13	0.27	0.01	0.29	2.19	0.03	1.92	0.04	0.05	0.00	191.82	4.01	4.91	0.10	661.46	13.82	16.92	0.35	F
Sample 120038	0.12	0.25	0.01	0.21	1.94	0.05	1.69	0.06	0.04	0.00	169.35	5.77	4.33	0.15	806.41	27.46	20.63	0.70	
Sample 120039	0.12	0.25	0.01	0.20	1.41	0.05	1.16	0.06	0.03	0.00	116.35	5.96	2.98	0.15	581.73	29.81	14.88	0.76	M
Sample 120040	0.13	0.27	0.01	0.17	1.16	0.01	0.88	0.02	0.02	0.00	88.50	1.77	2.26	0.05	520.57	10.41	13.31	0.27	
Sample 120041	0.12	0.25	0.01	0.32	1.11	0.05	0.86	0.06	0.02	0.00	85.81	5.57	2.19	0.14	268.16	17.41	6.86	0.45	F

K^+ Page 2

	Cotton Wool Mass (g)	CW K^+ Conc mg/L	CW SD	Mucus Mass (g)	Mucus K^+ Conc mg/L	Mucus SD	K^+ conc Mucus - CW mg/L	K^+ conc SD mg/L	K^+ conc Mucus - CW mmol/L	K^+ conc SD mmol/L	K^+ Conc (mg/L) X1000	K^+ Conc (mg/L) SD X1000	K^+ Conc (mmol/L) X1000	K^+ Conc (mmol/L) SD X1000	K^+ conc mg/L per g mucus	K^+ conc mg/L per g mucus SD	K^+ conc mmol/L per g mucus	K^+ conc mmol/L per g mucus SD	Sex
Sample 120042	0.13	0.27	0.01	0.21	1.45	0.03	1.18	0.03	0.03	0.00	118.28	3.46	3.03	0.09	563.23	16.47	14.41	0.42	
Sample 120043	0.15	0.31	0.01	0.20	1.10	0.02	0.79	0.03	0.02	0.00	78.68	2.52	2.01	0.06	393.41	12.62	10.06	0.32	F
Sample 120044	0.14	0.29	0.01	0.18	1.14	0.02	0.84	0.03	0.02	0.00	84.46	2.94	2.16	0.08	469.25	16.34	12.00	0.42	
Sample 120045	0.12	0.25	0.01	0.16	1.09	0.02	0.84	0.02	0.02	0.00	83.76	2.20	2.14	0.06	523.53	13.76	13.39	0.35	F
Sample 120046	0.14	0.29	0.01	0.10	0.86	0.02	0.56	0.03	0.01	0.00	56.36	2.62	1.44	0.07	563.63	26.18	14.42	0.67	
Sample 120047	0.12	0.25	0.01	0.20	1.25	0.05	1.00	0.05	0.03	0.00	100.26	5.20	2.56	0.13	501.32	26.00	12.82	0.67	M
Sample 120048	0.13	0.27	0.01	0.12	0.81	0.01	0.54	0.02	0.01	0.00	54.11	2.12	1.38	0.05	450.95	17.66	11.53	0.45	
Sample 120049	0.11	0.23	0.01	0.22	1.62	0.08	1.39	0.08	0.04	0.00	139.36	8.12	3.56	0.21	633.47	36.90	16.20	0.94	F
Sample 120050	0.13	0.27	0.01	0.40	2.72	0.03	2.45	0.04	0.06	0.00	244.84	3.77	6.26	0.10	612.10	9.42	15.66	0.24	
Sample 120051	0.11	0.23	0.01	0.15	0.92	0.04	0.69	0.05	0.02	0.00	69.04	4.67	1.77	0.12	460.26	31.16	11.77	0.80	M
Sample 120052	0.12	0.25	0.01	0.15	1.03	0.04	0.78	0.05	0.02	0.00	77.66	4.68	1.99	0.12	517.72	31.22	13.24	0.80	
Sample 120053	0.14	0.29	0.01	0.14	1.01	0.03	0.72	0.04	0.02	0.00	71.52	3.67	1.83	0.09	510.83	26.24	13.07	0.67	M
Sample 120054	0.13	0.27	0.01	0.36	2.10	0.06	1.83	0.06	0.05	0.00	182.69	6.49	4.67	0.17	507.47	18.04	12.98	0.46	
Sample 120055	0.12	0.25	0.01	0.40	3.12	0.11	2.87	0.12	0.07	0.00	287.39	11.67	7.35	0.30	718.47	29.17	18.38	0.75	F
Sample 120056	0.13	0.27	0.01	0.34	2.57	0.09	2.30	0.10	0.06	0.00	229.74	9.63	5.88	0.25	675.71	28.32	17.28	0.72	
Sample 120057	0.12	0.25	0.01	0.37	1.82	0.02	1.57	0.03	0.04	0.00	157.38	2.68	4.03	0.07	425.34	7.24	10.88	0.19	M
Sample 120058	0.13	0.27	0.01	0.52	2.65	0.07	2.37	0.07	0.06	0.00	237.40	7.33	6.07	0.19	456.55	14.10	11.68	0.36	
Sample 120059	0.13	0.27	0.01	0.43	2.13	0.06	1.85	0.06	0.05	0.00	185.37	6.45	4.74	0.17	431.08	15.01	11.03	0.38	M
Sample 120060	0.13	0.27	0.01	0.47	4.42	0.21	4.15	0.22	0.11	0.01	415.29	21.66	10.62	0.55	883.59	46.09	22.60	1.18	
Sample 120061	0.12	0.25	0.01	0.31	2.72	0.06	2.47	0.07	0.06	0.00	247.00	6.84	6.32	0.18	796.78	22.08	20.38	0.56	F
Sample 120062	0.13	0.27	0.01	0.32	2.97	0.02	2.70	0.02	0.07	0.00	269.58	2.44	6.89	0.06	842.44	7.63	21.55	0.20	
Sample 120063	0.11	0.23	0.01	0.27	1.79	0.03	1.56	0.03	0.04	0.00	155.88	3.29	3.99	0.08	577.34	12.19	14.77	0.31	M
Sample 120064	0.13	0.27	0.01	0.22	1.36	0.04	1.09	0.05	0.03	0.00	109.11	4.83	2.79	0.12	495.95	21.95	12.68	0.56	
Sample 120065	0.12	0.25	0.01	0.30	2.37	0.08	2.12	0.09	0.05	0.00	212.09	8.88	5.42	0.23	706.98	29.58	18.08	0.76	M
Sample 120066	0.13	0.27	0.01	0.35	2.59	0.09	2.32	0.10	0.06	0.00	231.77	10.11	5.93	0.26	662.20	28.89	16.94	0.74	
Sample 120067	0.11	0.23	0.01	0.17	2.39	0.04	2.16	0.04	0.06	0.00	215.85	4.47	5.52	0.11	1269.68	26.28	32.47	0.67	M
Sample 120068	0.13	0.27	0.01	0.16	1.11	0.02	0.84	0.03	0.02	0.00	83.95	3.07	2.15	0.08	524.68	19.21	13.42	0.49	

K^+ Page 3

	Cotton Wool Mass (g)	CW K^+ Conc mg/L	CW SD	Mucus Mass (g)	Mucus K^+ Conc mg/L	Mucus SD	K^+ conc Mucus - CW mg/L	K^+ conc SD mg/L	K^+ conc Mucus - CW mmol/L	K^+ conc SD mmol/L	K^+ Conc (mg/L) X1000	K^+ Conc (mg/L) SD X1000	K^+ Conc (mmol/L) X1000	K^+ Conc (mmol/L) SD X1000	K^+ conc mg/L per g mucus	K^+ conc mg/L per g mucus SD	K^+ conc mmol/L per g mucus	K^+ conc mmol/L per g mucus SD	Sex
Sample 120069	0.14	0.29	0.01	0.54	4.14	0.05	3.85	0.06	0.10	0.00	384.81	5.76	9.84	0.15	712.62	10.67	18.23	0.27	F
Sample 120070	0.13	0.27	0.01	0.60	2.45	0.04	2.18	0.05	0.06	0.00	218.10	5.17	5.58	0.13	363.51	8.62	9.30	0.22	
Sample 120071	0.12	0.25	0.01	0.15	1.34	0.02	1.09	0.02	0.03	0.00	108.72	2.27	2.78	0.06	724.77	15.13	18.54	0.39	F
Sample 120072	0.11	0.23	0.01	0.14	0.88	0.03	0.65	0.03	0.02	0.00	65.45	3.45	1.67	0.09	467.52	24.66	11.96	0.63	
Sample 120073	0.13	0.27	0.01	0.14	0.99	0.01	0.72	0.02	0.02	0.00	72.05	1.50	1.84	0.04	514.64	10.73	13.16	0.27	M
Sample 120074	0.13	0.27	0.01	0.19	1.07	0.02	0.80	0.03	0.02	0.00	79.61	2.93	2.04	0.07	418.99	15.40	10.72	0.39	
Sample 120075	0.13	0.27	0.01	0.32	2.39	0.02	2.11	0.03	0.05	0.00	211.40	3.21	5.41	0.08	660.63	10.03	16.90	0.26	F
Sample 120076	0.13	0.27	0.01	0.36	2.87	0.02	2.59	0.02	0.07	0.00	259.33	2.39	6.63	0.06	720.36	6.65	18.42	0.17	
Sample 120077	0.11	0.23	0.01	0.43	4.06	0.28	3.83	0.29	0.10	0.01	382.92	28.69	9.79	0.73	890.50	66.73	22.78	1.71	F
Sample 120078	0.12	0.25	0.01	0.47	4.29	0.10	4.04	0.10	0.10	0.00	403.92	10.32	10.33	0.26	859.41	21.97	21.98	0.56	
Sample 120079	0.12	0.25	0.01	0.57	5.75	0.21	5.50	0.21	0.14	0.01	550.05	21.34	14.07	0.55	964.99	37.44	24.68	0.96	M
Sample 120080	0.12	0.25	0.01	0.61	5.64	0.28	5.39	0.29	0.14	0.01	539.13	29.12	13.79	0.74	883.82	47.74	22.60	1.22	
Sample 120081	0.12	0.25	0.01	0.58	3.52	0.03	3.26	0.03	0.08	0.00	326.46	3.31	8.35	0.08	562.86	5.71	14.40	0.15	M
Sample 120082	0.12	0.25	0.01	1.07	5.99	0.39	5.74	0.40	0.15	0.01	573.65	40.07	14.67	1.02	536.12	37.45	13.71	0.96	
Sample 120083	0.12	0.25	0.01	0.51	3.79	0.11	3.54	0.12	0.09	0.00	353.97	12.11	9.05	0.31	694.05	23.74	17.75	0.61	M
Sample 120084	0.12	0.25	0.01	0.45	2.70	0.09	2.44	0.10	0.06	0.00	244.41	10.00	6.25	0.26	543.14	22.23	13.89	0.57	
Sample 120085	0.12	0.25	0.01	0.20	1.41	0.03	1.16	0.03	0.03	0.00	115.87	3.32	2.96	0.09	579.36	16.62	14.82	0.43	F
Sample 120086	0.13	0.27	0.01	0.18	1.22	0.02	0.95	0.02	0.02	0.00	95.06	2.49	2.43	0.06	528.11	13.82	13.51	0.35	
Sample 120087	0.11	0.23	0.01	0.29	1.83	0.04	1.60	0.05	0.04	0.00	159.63	4.56	4.08	0.12	550.43	15.73	14.08	0.40	M
Sample 120088	0.13	0.27	0.01	0.33	2.96	0.01	2.68	0.01	0.07	0.00	268.44	1.31	6.87	0.03	813.47	3.98	20.81	0.10	
Sample 120089	0.13	0.27	0.01	0.41	2.46	0.09	2.19	0.09	0.06	0.00	218.61	9.33	5.59	0.24	533.20	22.74	13.64	0.58	M
Sample 120090	0.12	0.25	0.01	0.41	3.68	0.13	3.43	0.14	0.09	0.00	342.94	14.00	8.77	0.36	836.44	34.15	21.39	0.87	
Sample 120091	0.12	0.25	0.01	0.32	2.39	0.05	2.14	0.06	0.05	0.00	214.09	6.06	5.48	0.16	669.02	18.95	17.11	0.48	F
Sample 120092	0.13	0.27	0.01	0.43	2.71	0.04	2.44	0.05	0.06	0.00	244.11	4.78	6.24	0.12	567.69	11.13	14.52	0.28	
Sample 120093	0.12	0.25	0.01	0.17	0.96	0.01	0.71	0.01	0.02	0.00	70.90	1.44	1.81	0.04	417.08	8.47	10.67	0.22	F
Sample 120094	0.12	0.25	0.01	0.30	1.95	0.02	1.70	0.03	0.04	0.00	169.66	2.54	4.34	0.06	565.54	8.46	14.46	0.22	

(c) Ca^{2+}

	Cotton Wool Mass (g)	CW Ca^{2+} Conc mg/L	CW SD	Mucus Mass (g)	Mucus Ca^{2+} Conc mg/L	Mucus SD	Ca^{2+} conc Mucus - CW mg/L	Ca^{2+} conc SD mg/L	Ca^{2+} conc Mucus - CW mmol/L	Ca^{2+} conc SD mmol/L	Ca^{2+} Conc (mg/L) X1000	Ca^{2+} Conc (mg/L) SD X1000	Ca^{2+} Conc (mmol/L) X1000	Ca^{2+} Conc (mmol/L) SD X1000	Ca^{2+} conc mg/L per g mucus	Ca^{2+} conc mg/L per g mucus SD	Ca^{2+} conc mmol/L per g mucus	Ca^{2+} conc mmol/L per g mucus SD	Sex
Sample 120015	0.15	0.44	0.01	0.38	0.02	0.00	-0.42	0.01	-0.01	0.00	-42.49	1.42	-1.06	0.04	-111.82	3.72	-2.79	0.09	F
Sample 120016	0.10	0.30	0.01	0.38	1.20	0.00	0.91	0.01	0.02	0.00	90.87	1.17	2.27	0.03	239.13	3.09	5.97	0.08	
Sample 120017	0.13	0.38	0.01	0.74	1.57	0.02	1.19	0.03	0.03	0.00	118.83	2.91	2.96	0.07	160.57	3.93	4.01	0.10	F
Sample 120018	0.10	0.30	0.01	0.75	1.78	0.01	1.48	0.02	0.04	0.00	148.10	2.15	3.70	0.05	197.46	2.86	4.93	0.07	
Sample 120019	0.12	0.35	0.01	0.39	1.41	0.03	1.05	0.04	0.03	0.00	105.37	4.40	2.63	0.11	270.18	11.27	6.74	0.28	M
Sample 120020	0.22	0.65	0.02	0.33	1.83	0.03	1.18	0.04	0.03	0.00	117.72	4.39	2.94	0.11	356.74	13.29	8.90	0.33	
Sample 120021	0.13	0.38	0.01	0.39	1.26	0.02	0.88	0.03	0.02	0.00	87.90	2.94	2.19	0.07	225.39	7.53	5.62	0.19	M
Sample 120022	0.13	0.38	0.01	0.46	1.44	0.03	1.06	0.04	0.03	0.00	105.65	4.02	2.64	0.10	229.66	8.74	5.73	0.22	
Sample 120023	0.11	0.32	0.01	0.33	1.26	0.03	0.94	0.04	0.02	0.00	93.76	3.52	2.34	0.09	284.12	10.65	7.09	0.27	F
Sample 120024	0.14	0.41	0.01	0.33	1.21	0.02	0.80	0.03	0.02	0.00	80.08	2.76	2.00	0.07	242.66	8.35	6.05	0.21	
Sample 120025	0.11	0.32	0.01	0.23	0.72	0.01	0.40	0.02	0.01	0.00	39.73	1.99	0.99	0.05	172.76	8.63	4.31	0.22	M
Sample 120026	0.11	0.32	0.01	0.31	1.01	0.03	0.68	0.04	0.02	0.00	68.40	3.59	1.71	0.09	220.65	11.59	5.51	0.29	
Sample 120027	0.12	0.35	0.01	0.62	1.30	0.03	0.95	0.04	0.02	0.00	94.81	3.53	2.37	0.09	152.91	5.69	3.82	0.14	M
Sample 120028	0.13	0.38	0.01	0.51	2.21	0.06	1.83	0.07	0.05	0.00	182.72	7.36	4.56	0.18	358.28	14.43	8.94	0.36	
Sample 120029	0.12	0.35	0.01	0.39	1.16	0.03	0.80	0.04	0.02	0.00	80.22	3.66	2.00	0.09	205.68	9.39	5.13	0.23	F
Sample 120030	0.12	0.35	0.01	0.32	1.06	0.01	0.70	0.02	0.02	0.00	70.16	1.79	1.75	0.04	219.26	5.58	5.47	0.14	
Sample 120031	0.12	0.35	0.01	0.18	0.83	0.00	0.47	0.01	0.01	0.00	47.29	0.99	1.18	0.02	262.74	5.49	6.56	0.14	M
Sample 120032	0.13	0.38	0.01	0.20	0.89	0.00	0.51	0.01	0.01	0.00	50.93	1.02	1.27	0.03	254.64	5.09	6.35	0.13	
Sample 120033	0.14	0.41	0.01	0.27	2.65	0.03	2.24	0.04	0.06	0.00	223.64	4.08	5.58	0.10	828.30	15.10	20.67	0.38	F
Sample 120034	0.12	0.35	0.01	0.22	0.86	0.00	0.50	0.01	0.01	0.00	50.39	1.05	1.26	0.03	229.07	4.79	5.72	0.12	
Sample 120035	0.13	0.38	0.01	0.50	13.80	1.03	13.41	1.04	0.33	0.03	1341.36	104.01	33.47	2.60	2682.73	208.03	66.94	5.19	F
Sample 120036	0.12	0.35	0.01	0.37	0.92	0.01	0.56	0.02	0.01	0.00	56.09	2.36	1.40	0.06	151.59	6.39	3.78	0.16	
Sample 120037	0.13	0.38	0.01	0.29	1.00	0.03	0.61	0.04	0.02	0.00	61.26	3.59	1.53	0.09	211.25	12.38	5.27	0.31	F
Sample 120038	0.12	0.35	0.01	0.21	0.84	0.00	0.48	0.01	0.01	0.00	48.06	1.28	1.20	0.03	228.86	6.09	5.71	0.15	
Sample 120039	0.12	0.35	0.01	0.20	0.85	0.02	0.49	0.03	0.01	0.00	49.42	2.61	1.23	0.07	247.10	13.03	6.17	0.33	M
Sample 120040	0.13	0.38	0.01	0.17	0.90	0.02	0.51	0.03	0.01	0.00	51.11	2.69	1.28	0.07	300.63	15.85	7.50	0.40	
Sample 120041	0.12	0.35	0.01	0.32	7.76	0.13	7.40	0.13	0.18	0.00	740.42	13.45	18.47	0.34	2313.81	42.02	57.73	1.05	F

Ca^{2+} Page 2

	Cotton Wool Mass (g)	CW Ca^{2+} Conc mg/L	CW SD	Mucus Mass (g)	Mucus Ca^{2+} Conc mg/L	Mucus SD	Ca^{2+} conc Mucus - CW mg/L	Ca^{2+} conc SD mg/L	Ca^{2+} conc Mucus - CW mmol/L	Ca^{2+} conc SD mmol/L	Ca^{2+} Conc (mg/L) X1000	Ca^{2+} Conc (mg/L) SD X1000	Ca^{2+} Conc (mmol/L) X1000	Ca^{2+} Conc (mmol/L) SD X1000	Ca^{2+} conc mg/L per g mucus	Ca^{2+} conc mg/L per g mucus SD	Ca^{2+} conc mmol/L per g mucus	Ca^{2+} conc mmol/L per g mucus SD	Sex
Sample 120042	0.13	0.38	0.01	0.21	2.57	0.01	2.18	0.02	0.05	0.00	218.49	2.35	5.45	0.06	1040.44	11.20	25.96	0.28	
Sample 120043	0.15	0.44	0.01	0.20	0.91	0.03	0.46	0.04	0.01	0.00	46.35	4.43	1.16	0.11	231.76	22.13	5.78	0.55	F
Sample 120044	0.14	0.41	0.01	0.18	0.90	0.02	0.49	0.03	0.01	0.00	49.02	3.45	1.22	0.09	272.36	19.17	6.80	0.48	
Sample 120045	0.12	0.35	0.01	0.16	0.81	0.01	0.46	0.02	0.01	0.00	45.68	2.01	1.14	0.05	285.48	12.58	7.12	0.31	F
Sample 120046	0.14	0.41	0.01	0.10	1.82	0.02	1.41	0.03	0.04	0.00	140.97	3.31	3.52	0.08	1409.74	33.08	35.17	0.83	
Sample 120047	0.12	0.35	0.01	0.20	0.93	0.01	0.58	0.02	0.01	0.00	57.96	2.21	1.45	0.06	289.80	11.05	7.23	0.28	M
Sample 120048	0.13	0.38	0.01	0.12	0.81	0.03	0.43	0.04	0.01	0.00	42.59	3.97	1.06	0.10	354.95	33.10	8.86	0.83	
Sample 120049	0.11	0.32	0.01	0.22	1.21	0.01	0.88	0.02	0.02	0.00	88.17	2.06	2.20	0.05	400.77	9.37	10.00	0.23	F
Sample 120050	0.13	0.38	0.01	0.40	1.23	0.07	0.85	0.08	0.02	0.00	85.06	7.91	2.12	0.20	212.64	19.77	5.31	0.49	
Sample 120051	0.11	0.32	0.01	0.15	0.75	0.01	0.43	0.02	0.01	0.00	42.58	2.17	1.06	0.05	283.87	14.44	7.08	0.36	M
Sample 120052	0.12	0.35	0.01	0.15	0.84	0.00	0.49	0.01	0.01	0.00	48.63	1.10	1.21	0.03	324.17	7.35	8.09	0.18	
Sample 120053	0.14	0.41	0.01	0.14	0.87	0.02	0.45	0.03	0.01	0.00	45.31	3.05	1.13	0.08	323.62	21.76	8.07	0.54	M
Sample 120054	0.13	0.38	0.01	0.36	1.20	0.04	0.82	0.05	0.02	0.00	81.54	4.73	2.03	0.12	226.51	13.14	5.65	0.33	
Sample 120055	0.12	0.35	0.01	0.40	1.01	0.02	0.65	0.02	0.02	0.00	65.06	2.48	1.62	0.06	162.65	6.21	4.06	0.15	F
Sample 120056	0.13	0.38	0.01	0.34	1.04	0.02	0.66	0.03	0.02	0.00	65.59	3.23	1.64	0.08	192.93	9.50	4.81	0.24	
Sample 120057	0.12	0.35	0.01	0.37	1.02	0.00	0.67	0.01	0.02	0.00	66.63	1.21	1.66	0.03	180.07	3.26	4.49	0.08	M
Sample 120058	0.13	0.38	0.01	0.52	1.15	0.01	0.77	0.02	0.02	0.00	76.98	1.66	1.92	0.04	148.04	3.19	3.69	0.08	
Sample 120059	0.13	0.38	0.01	0.43	1.05	0.02	0.67	0.03	0.02	0.00	67.03	3.46	1.67	0.09	155.88	8.05	3.89	0.20	M
Sample 120060	0.13	0.38	0.01	0.47	1.68	0.02	1.30	0.03	0.03	0.00	129.70	3.43	3.24	0.09	275.95	7.31	6.89	0.18	
Sample 120061	0.12	0.35	0.01	0.31	1.30	0.02	0.94	0.03	0.02	0.00	94.06	3.16	2.35	0.08	303.42	10.19	7.57	0.25	F
Sample 120062	0.13	0.38	0.01	0.32	1.78	0.05	1.40	0.06	0.03	0.00	139.85	6.03	3.49	0.15	437.03	18.83	10.90	0.47	
Sample 120063	0.11	0.32	0.01	0.27	0.89	0.01	0.57	0.02	0.01	0.00	56.89	1.79	1.42	0.04	210.70	6.61	5.26	0.16	M
Sample 120064	0.13	0.38	0.01	0.22	0.88	0.02	0.49	0.03	0.01	0.00	49.47	3.04	1.23	0.08	224.85	13.82	5.61	0.34	
Sample 120065	0.12	0.35	0.01	0.30	0.99	0.03	0.64	0.04	0.02	0.00	63.68	3.78	1.59	0.09	212.26	12.59	5.30	0.31	M
Sample 120066	0.13	0.38	0.01	0.35	1.05	0.00	0.66	0.01	0.02	0.00	66.16	1.40	1.65	0.03	189.02	3.99	4.72	0.10	
Sample 120067	0.11	0.32	0.01	0.17	1.37	0.03	1.04	0.04	0.03	0.00	104.12	3.97	2.60	0.10	612.46	23.33	15.28	0.58	M
Sample 120068	0.13	0.38	0.01	0.16	0.66	0.02	0.28	0.03	0.01	0.00	27.98	3.36	0.70	0.08	174.87	20.99	4.36	0.52	

Ca^{2+} Page 3

	Cotton Wool Mass (g)	CW Ca^{2+} Conc mg/L	CW SD	Mucus Mass (g)	Mucus Ca^{2+} Conc mg/L	Mucus SD	Ca^{2+} conc Mucus - CW mg/L	Ca^{2+} conc SD mg/L	Ca^{2+} conc Mucus - CW mmol/L	Ca^{2+} conc SD mmol/L	Ca^{2+} Conc (mg/L) X1000	Ca^{2+} Conc (mg/L) SD X1000	Ca^{2+} Conc (mmol/L) X1000	Ca^{2+} Conc (mmol/L) SD X1000	Ca^{2+} conc mg/L per g mucus	Ca^{2+} conc mg/L per g mucus SD	Ca^{2+} conc mmol/L per g mucus	Ca^{2+} conc mmol/L per g mucus SD	Sex
Sample 120069	0.14	0.41	0.01	0.54	0.89	0.02	0.47	0.03	0.01	0.00	47.18	2.66	1.18	0.07	87.37	4.93	2.18	0.12	F
Sample 120070	0.13	0.38	0.01	0.60	0.47	0.01	0.09	0.01	0.00	0.00	9.08	1.50	0.23	0.04	15.14	2.50	0.38	0.06	
Sample 120071	0.12	0.35	0.01	0.15	0.57	0.04	0.21	0.05	0.01	0.00	21.22	4.52	0.53	0.11	141.44	30.14	3.53	0.75	F
Sample 120072	0.11	0.32	0.01	0.14	0.48	0.01	0.16	0.02	0.00	0.00	15.66	2.25	0.39	0.06	111.84	16.06	2.79	0.40	
Sample 120073	0.13	0.38	0.01	0.14	0.79	0.04	0.41	0.05	0.01	0.00	40.80	4.53	1.02	0.11	291.43	32.38	7.27	0.81	M
Sample 120074	0.13	0.38	0.01	0.19	0.57	0.01	0.18	0.02	0.00	0.00	18.29	1.78	0.46	0.04	96.25	9.39	2.40	0.23	
Sample 120075	0.13	0.38	0.01	0.32	0.79	0.01	0.41	0.02	0.01	0.00	41.00	1.74	1.02	0.04	128.14	5.45	3.20	0.14	F
Sample 120076	0.13	0.38	0.01	0.36	0.87	0.04	0.48	0.05	0.01	0.00	48.16	5.10	1.20	0.13	133.77	14.18	3.34	0.35	
Sample 120077	0.11	0.32	0.01	0.43	1.09	0.02	0.77	0.03	0.02	0.00	76.79	2.62	1.92	0.07	178.57	6.08	4.46	0.15	F
Sample 120078	0.12	0.35	0.01	0.47	0.89	0.01	0.53	0.02	0.01	0.00	53.09	1.73	1.32	0.04	112.95	3.68	2.82	0.09	
Sample 120079	0.12	0.35	0.01	0.57	1.76	0.04	1.40	0.05	0.04	0.00	140.47	4.61	3.50	0.12	246.44	8.09	6.15	0.20	M
Sample 120080	0.12	0.35	0.01	0.61	1.20	0.04	0.85	0.05	0.02	0.00	84.73	4.77	2.11	0.12	138.90	7.82	3.47	0.20	
Sample 120081	0.12	0.35	0.01	0.58	0.95	0.01	0.59	0.02	0.01	0.00	59.09	2.16	1.47	0.05	101.87	3.72	2.54	0.09	M
Sample 120082	0.12	0.35	0.01	1.07	1.47	0.03	1.11	0.04	0.03	0.00	111.47	4.00	2.78	0.10	104.18	3.73	2.60	0.09	
Sample 120083	0.12	0.35	0.01	0.51	0.98	0.05	0.63	0.05	0.02	0.00	62.98	5.47	1.57	0.14	123.50	10.73	3.08	0.27	M
Sample 120084	0.12	0.35	0.01	0.45	1.02	0.02	0.67	0.03	0.02	0.00	66.98	3.37	1.67	0.08	148.85	7.49	3.71	0.19	
Sample 120085	0.12	0.35	0.01	0.20	0.62	0.02	0.27	0.02	0.01	0.00	26.57	2.49	0.66	0.06	132.87	12.44	3.32	0.31	F
Sample 120086	0.13	0.38	0.01	0.18	0.69	0.02	0.30	0.03	0.01	0.00	30.18	2.88	0.75	0.07	167.69	16.02	4.18	0.40	
Sample 120087	0.11	0.32	0.01	0.29	0.82	0.02	0.49	0.02	0.01	0.00	49.37	2.45	1.23	0.06	170.26	8.47	4.25	0.21	M
Sample 120088	0.13	0.38	0.01	0.33	0.91	0.01	0.52	0.02	0.01	0.00	52.13	1.86	1.30	0.05	157.98	5.64	3.94	0.14	
Sample 120089	0.13	0.38	0.01	0.41	0.84	0.02	0.46	0.03	0.01	0.00	45.70	2.78	1.14	0.07	111.46	6.79	2.78	0.17	M
Sample 120090	0.12	0.35	0.01	0.41	0.99	0.03	0.64	0.03	0.02	0.00	63.70	3.43	1.59	0.09	155.37	8.37	3.88	0.21	
Sample 120091	0.12	0.35	0.01	0.32	0.76	0.01	0.40	0.02	0.01	0.00	40.11	1.95	1.00	0.05	125.35	6.08	3.13	0.15	F
Sample 120092	0.13	0.38	0.01	0.43	0.82	0.03	0.44	0.04	0.01	0.00	43.59	3.55	1.09	0.09	101.38	8.24	2.53	0.21	
Sample 120093	0.12	0.35	0.01	0.17	0.84	0.04	0.49	0.04	0.01	0.00	48.83	4.46	1.22	0.11	287.24	26.22	7.17	0.65	F
Sample 120094	0.12	0.35	0.01	0.30	0.71	0.02	0.36	0.03	0.01	0.00	35.57	2.79	0.89	0.07	118.58	9.30	2.96	0.23	

(d) Mg^{2+}

	Cotton Wool Mass (g)	CW Mg^{2+} Conc mg/L	CW SD	Mucus Mass (g)	Mucus Mg^{2+} Conc mg/L	Mucus SD	Mg^{2+} conc Mucus - CW mg/L	Mg^{2+} conc SD mg/L	Mg^{2+} conc Mucus - CW mmol/L	Mg^{2+} conc SD mmol/L	Mg^{2+} Conc (mg/L) X1000	Mg^{2+} Conc (mg/L) SD X1000	Mg^{2+} Conc (mmol/L) X1000	Mg^{2+} Conc (mmol/L) SD X1000	Mg^{2+} conc mg/L per g mucus	Mg^{2+} conc mg/L per g mucus SD	Mg^{2+} conc mmol/L per g mucus	Mg^{2+} conc mmol/L per g mucus SD	Sex
Sample 120015	0.15	0.16	0.00	0.38	0.18	0.00	0.02	0.01	0.00	0.00	1.93	0.69	0.08	0.03	5.07	1.82	0.21	0.07	F
Sample 120016	0.10	0.11	0.00	0.38	0.17	0.00	0.07	0.00	0.00	0.00	6.71	0.23	0.28	0.01	17.66	0.60	0.73	0.02	
Sample 120017	0.13	0.14	0.00	0.74	0.19	0.01	0.05	0.01	0.00	0.00	5.09	0.84	0.21	0.03	6.88	1.14	0.28	0.05	F
Sample 120018	0.10	0.11	0.00	0.75	0.22	0.00	0.12	0.00	0.00	0.00	11.70	0.28	0.48	0.01	15.61	0.37	0.64	0.02	
Sample 120019	0.12	0.13	0.00	0.39	0.20	0.00	0.08	0.00	0.00	0.00	7.59	0.49	0.31	0.02	19.47	1.26	0.80	0.05	M
Sample 120020	0.22	0.23	0.00	0.33	0.21	0.01	-0.02	0.01	0.00	0.00	-1.75	0.91	-0.07	0.04	-5.31	2.75	-0.22	0.11	
Sample 120021	0.13	0.14	0.00	0.39	0.18	0.00	0.05	0.01	0.00	0.00	4.59	0.54	0.19	0.02	11.77	1.38	0.48	0.06	M
Sample 120022	0.13	0.14	0.00	0.46	0.21	0.01	0.08	0.01	0.00	0.00	7.66	0.90	0.32	0.04	16.65	1.96	0.68	0.08	
Sample 120023	0.11	0.12	0.00	0.33	0.21	0.00	0.10	0.00	0.00	0.00	9.55	0.44	0.39	0.02	28.93	1.33	1.19	0.05	F
Sample 120024	0.14	0.15	0.00	0.33	0.21	0.00	0.07	0.01	0.00	0.00	6.70	0.69	0.28	0.03	20.30	2.08	0.84	0.09	
Sample 120025	0.11	0.12	0.00	0.23	0.14	0.00	0.02	0.00	0.00	0.00	2.32	0.34	0.10	0.01	10.10	1.49	0.42	0.06	M
Sample 120026	0.11	0.12	0.00	0.31	0.19	0.00	0.07	0.00	0.00	0.00	7.04	0.47	0.29	0.02	22.71	1.51	0.93	0.06	
Sample 120027	0.12	0.13	0.00	0.62	0.24	0.00	0.11	0.01	0.00	0.00	11.08	0.58	0.46	0.02	17.87	0.93	0.74	0.04	M
Sample 120028	0.13	0.14	0.00	0.51	0.25	0.00	0.11	0.01	0.00	0.00	11.39	0.60	0.47	0.02	22.34	1.17	0.92	0.05	
Sample 120029	0.12	0.13	0.00	0.39	0.21	0.00	0.08	0.01	0.00	0.00	7.92	0.56	0.33	0.02	20.30	1.43	0.84	0.06	F
Sample 120030	0.12	0.13	0.00	0.32	0.19	0.00	0.06	0.00	0.00	0.00	5.86	0.49	0.24	0.02	18.31	1.53	0.75	0.06	
Sample 120031	0.12	0.13	0.00	0.18	0.16	0.00	0.04	0.01	0.00	0.00	3.63	0.56	0.15	0.02	20.19	3.10	0.83	0.13	M
Sample 120032	0.13	0.14	0.00	0.20	0.18	0.01	0.04	0.01	0.00	0.00	3.96	0.80	0.16	0.03	19.80	4.02	0.81	0.17	
Sample 120033	0.14	0.15	0.00	0.27	0.22	0.00	0.07	0.01	0.00	0.00	6.79	0.61	0.28	0.03	25.14	2.27	1.03	0.09	F
Sample 120034	0.12	0.13	0.00	0.22	0.16	0.01	0.03	0.01	0.00	0.00	3.23	0.93	0.13	0.04	14.67	4.22	0.60	0.17	
Sample 120035	0.13	0.14	0.00	0.50	0.37	0.01	0.24	0.01	0.01	0.00	23.55	0.95	0.97	0.04	47.10	1.91	1.94	0.08	F
Sample 120036	0.12	0.13	0.00	0.37	0.19	0.00	0.07	0.01	0.00	0.00	6.82	0.57	0.28	0.02	18.43	1.54	0.76	0.06	
Sample 120037	0.13	0.14	0.00	0.29	0.18	0.00	0.05	0.00	0.00	0.00	4.72	0.47	0.19	0.02	16.26	1.63	0.67	0.07	F
Sample 120038	0.12	0.13	0.00	0.21	0.17	0.00	0.04	0.00	0.00	0.00	4.23	0.49	0.17	0.02	20.13	2.35	0.83	0.10	
Sample 120039	0.12	0.13	0.00	0.20	0.19	0.00	0.06	0.01	0.00	0.00	5.86	0.56	0.24	0.02	29.28	2.82	1.20	0.12	M
Sample 120040	0.13	0.14	0.00	0.17	0.19	0.00	0.05	0.00	0.00	0.00	4.82	0.33	0.20	0.01	28.38	1.91	1.17	0.08	
Sample 120041	0.12	0.13	0.00	0.32	0.22	0.00	0.10	0.01	0.00	0.00	9.73	0.54	0.40	0.02	30.40	1.69	1.25	0.07	F

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	Cotton Wool Mass (g)	CW Mg^{2+} Conc mg/L	CW SD	Mucus Mass (g)	Mucus Mg^{2+} Conc mg/L	Mucus SD	Mg^{2+} conc Mucus - CW mg/L	Mg^{2+} conc SD mg/L	Mg^{2+} conc Mucus - CW mmol/L	Mg^{2+} conc SD mmol/L	Mg^{2+} Conc (mg/L) X1000	Mg^{2+} Conc (mg/L) SD X1000	Mg^{2+} Conc (mmol/L) X1000	Mg^{2+} Conc (mmol/L) SD X1000	Mg^{2+} conc mg/L per g mucus	Mg^{2+} conc mg/L per g mucus SD	Mg^{2+} conc mmol/L per g mucus	Mg^{2+} conc mmol/L per g mucus SD	Sex
Sample 120042	0.13	0.14	0.00	0.21	0.21	0.00	0.08	0.01	0.00	0.00	7.66	0.60	0.32	0.02	36.47	2.85	1.50	0.12	
Sample 120043	0.15	0.16	0.00	0.20	0.20	0.00	0.04	0.01	0.00	0.00	4.12	0.71	0.17	0.03	20.61	3.55	0.85	0.15	F
Sample 120044	0.14	0.15	0.00	0.18	0.20	0.01	0.05	0.01	0.00	0.00	4.94	1.03	0.20	0.04	27.47	5.72	1.13	0.24	
Sample 120045	0.12	0.13	0.00	0.16	0.17	0.00	0.04	0.00	0.00	0.00	4.44	0.36	0.18	0.01	27.75	2.22	1.14	0.09	F
Sample 120046	0.14	0.15	0.00	0.10	0.19	0.00	0.04	0.00	0.00	0.00	4.19	0.40	0.17	0.02	41.88	4.04	1.72	0.17	
Sample 120047	0.12	0.13	0.00	0.20	0.19	0.00	0.06	0.00	0.00	0.00	6.34	0.36	0.26	0.01	31.69	1.81	1.30	0.07	M
Sample 120048	0.13	0.14	0.00	0.12	0.18	0.01	0.04	0.01	0.00	0.00	4.29	0.79	0.18	0.03	35.76	6.58	1.47	0.27	
Sample 120049	0.11	0.12	0.00	0.22	0.20	0.01	0.09	0.01	0.00	0.00	8.64	0.98	0.36	0.04	39.25	4.46	1.61	0.18	F
Sample 120050	0.13	0.14	0.00	0.40	0.22	0.00	0.08	0.01	0.00	0.00	8.29	0.57	0.34	0.02	20.73	1.43	0.85	0.06	
Sample 120051	0.11	0.12	0.00	0.15	0.17	0.00	0.05	0.00	0.00	0.00	5.39	0.32	0.22	0.01	35.95	2.15	1.48	0.09	M
Sample 120052	0.12	0.13	0.00	0.15	0.18	0.00	0.06	0.01	0.00	0.00	5.52	0.60	0.23	0.02	36.82	4.03	1.51	0.17	
Sample 120053	0.14	0.15	0.00	0.14	0.19	0.01	0.04	0.01	0.00	0.00	4.45	0.81	0.18	0.03	31.76	5.79	1.31	0.24	M
Sample 120054	0.13	0.14	0.00	0.36	0.24	0.00	0.10	0.00	0.00	0.00	10.11	0.38	0.42	0.02	28.10	1.05	1.16	0.04	
Sample 120055	0.12	0.13	0.00	0.40	0.19	0.00	0.06	0.01	0.00	0.00	6.29	0.66	0.26	0.03	15.72	1.66	0.65	0.07	F
Sample 120056	0.13	0.14	0.00	0.34	0.21	0.00	0.07	0.00	0.00	0.00	7.25	0.42	0.30	0.02	21.32	1.24	0.88	0.05	
Sample 120057	0.12	0.13	0.00	0.37	0.20	0.00	0.07	0.00	0.00	0.00	7.13	0.44	0.29	0.02	19.28	1.20	0.79	0.05	M
Sample 120058	0.13	0.14	0.00	0.52	0.22	0.00	0.08	0.00	0.00	0.00	7.88	0.28	0.32	0.01	15.16	0.54	0.62	0.02	
Sample 120059	0.13	0.14	0.00	0.43	0.22	0.00	0.08	0.01	0.00	0.00	8.26	0.69	0.34	0.03	19.20	1.59	0.79	0.07	M
Sample 120060	0.13	0.14	0.00	0.47	0.33	0.00	0.19	0.00	0.01	0.00	18.82	0.43	0.77	0.02	40.05	0.92	1.65	0.04	
Sample 120061	0.12	0.13	0.00	0.31	0.22	0.00	0.09	0.01	0.00	0.00	9.08	0.64	0.37	0.03	29.29	2.06	1.21	0.08	F
Sample 120062	0.13	0.14	0.00	0.32	0.28	0.01	0.14	0.01	0.01	0.00	13.85	0.95	0.57	0.04	43.28	2.97	1.78	0.12	
Sample 120063	0.11	0.12	0.00	0.27	0.19	0.00	0.08	0.01	0.00	0.00	7.75	0.54	0.32	0.02	28.72	2.00	1.18	0.08	M
Sample 120064	0.13	0.14	0.00	0.22	0.19	0.00	0.05	0.00	0.00	0.00	5.28	0.41	0.22	0.02	23.98	1.88	0.99	0.08	
Sample 120065	0.12	0.13	0.00	0.30	0.21	0.00	0.09	0.00	0.00	0.00	8.57	0.45	0.35	0.02	28.58	1.49	1.18	0.06	M
Sample 120066	0.13	0.14	0.00	0.35	0.22	0.01	0.08	0.01	0.00	0.00	7.93	0.79	0.33	0.03	22.64	2.27	0.93	0.09	
Sample 120067	0.11	0.12	0.00	0.17	0.29	0.00	0.17	0.00	0.01	0.00	17.44	0.49	0.72	0.02	102.56	2.91	4.22	0.12	M
Sample 120068	0.13	0.14	0.00	0.16	0.18	0.00	0.04	0.00	0.00	0.00	3.88	0.28	0.16	0.01	24.25	1.72	1.00	0.07	

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	Cotton Wool Mass (g)	CW Mg^{2+} Conc mg/L	CW SD	Mucus Mass (g)	Mucus Mg^{2+} Conc mg/L	Mucus SD	Mg^{2+} conc Mucus - CW mg/L	Mg^{2+} conc SD mg/L	Mg^{2+} conc Mucus - CW mmol/L	Mg^{2+} conc SD mmol/L	Mg^{2+} Conc (mg/L) X1000	Mg^{2+} Conc (mg/L) SD X1000	Mg^{2+} Conc (mmol/L) X1000	Mg^{2+} Conc (mmol/L) SD X1000	Mg^{2+} conc mg/L per g mucus	Mg^{2+} conc mg/L per g mucus SD	Mg^{2+} conc mmol/L per g mucus	Mg^{2+} conc mmol/L per g mucus SD	Sex
Sample 120069	0.14	0.15	0.00	0.54	0.21	0.01	0.07	0.01	0.00	0.00	6.52	0.75	0.27	0.03	12.08	1.39	0.50	0.06	F
Sample 120070	0.13	0.14	0.00	0.60	0.10	0.00	-0.04	0.00	0.00	0.00	-4.04	0.36	-0.17	0.01	-6.73	0.60	-0.28	0.02	
Sample 120071	0.12	0.13	0.00	0.15	0.16	0.00	0.04	0.01	0.00	0.00	3.70	0.55	0.15	0.02	24.69	3.66	1.02	0.15	F
Sample 120072	0.11	0.12	0.00	0.14	0.14	0.00	0.03	0.00	0.00	0.00	2.89	0.48	0.12	0.02	20.68	3.40	0.85	0.14	
Sample 120073	0.13	0.14	0.00	0.14	0.16	0.00	0.02	0.01	0.00	0.00	2.12	0.70	0.09	0.03	15.15	5.03	0.62	0.21	M
Sample 120074	0.13	0.14	0.00	0.19	0.16	0.00	0.02	0.00	0.00	0.00	1.99	0.31	0.08	0.01	10.46	1.64	0.43	0.07	
Sample 120075	0.13	0.14	0.00	0.32	0.17	0.00	0.03	0.01	0.00	0.00	3.41	0.72	0.14	0.03	10.66	2.26	0.44	0.09	F
Sample 120076	0.13	0.14	0.00	0.36	0.18	0.00	0.05	0.00	0.00	0.00	4.59	0.37	0.19	0.02	12.74	1.04	0.52	0.04	
Sample 120077	0.11	0.12	0.00	0.43	0.20	0.00	0.08	0.01	0.00	0.00	7.90	0.52	0.32	0.02	18.37	1.21	0.76	0.05	F
Sample 120078	0.12	0.13	0.00	0.47	0.18	0.00	0.06	0.01	0.00	0.00	5.51	0.52	0.23	0.02	11.72	1.11	0.48	0.05	
Sample 120079	0.12	0.13	0.00	0.57	0.24	0.01	0.11	0.01	0.00	0.00	11.27	0.78	0.46	0.03	19.77	1.37	0.81	0.06	M
Sample 120080	0.12	0.13	0.00	0.61	0.22	0.00	0.10	0.01	0.00	0.00	9.81	0.69	0.40	0.03	16.07	1.13	0.66	0.05	
Sample 120081	0.12	0.13	0.00	0.58	0.19	0.00	0.06	0.00	0.00	0.00	5.87	0.48	0.24	0.02	10.12	0.82	0.42	0.03	M
Sample 120082	0.12	0.13	0.00	1.07	0.25	0.01	0.12	0.01	0.00	0.00	12.13	0.82	0.50	0.03	11.34	0.76	0.47	0.03	
Sample 120083	0.12	0.13	0.00	0.51	0.19	0.01	0.07	0.01	0.00	0.00	6.54	0.80	0.27	0.03	12.81	1.57	0.53	0.06	M
Sample 120084	0.12	0.13	0.00	0.45	0.20	0.01	0.07	0.01	0.00	0.00	7.44	1.02	0.31	0.04	16.52	2.27	0.68	0.09	
Sample 120085	0.12	0.13	0.00	0.20	0.16	0.00	0.03	0.00	0.00	0.00	3.35	0.29	0.14	0.01	16.77	1.43	0.69	0.06	F
Sample 120086	0.13	0.14	0.00	0.18	0.18	0.00	0.04	0.01	0.00	0.00	3.98	0.53	0.16	0.02	22.12	2.92	0.91	0.12	
Sample 120087	0.11	0.12	0.00	0.29	0.21	0.00	0.10	0.01	0.00	0.00	9.79	0.63	0.40	0.03	33.75	2.18	1.39	0.09	M
Sample 120088	0.13	0.14	0.00	0.33	0.20	0.00	0.06	0.00	0.00	0.00	6.25	0.50	0.26	0.02	18.95	1.51	0.78	0.06	
Sample 120089	0.13	0.14	0.00	0.41	0.18	0.00	0.05	0.01	0.00	0.00	4.59	0.52	0.19	0.02	11.19	1.26	0.46	0.05	M
Sample 120090	0.12	0.13	0.00	0.41	0.20	0.01	0.07	0.01	0.00	0.00	7.00	0.74	0.29	0.03	17.08	1.82	0.70	0.07	
Sample 120091	0.12	0.13	0.00	0.32	0.18	0.00	0.05	0.00	0.00	0.00	4.98	0.47	0.21	0.02	15.57	1.47	0.64	0.06	F
Sample 120092	0.13	0.14	0.00	0.43	0.12	0.11	-0.01	0.11	0.00	0.00	-1.31	10.80	-0.05	0.44	-3.04	25.13	-0.13	1.03	
Sample 120093	0.12	0.13	0.00	0.17	0.15	0.00	0.03	0.00	0.00	0.00	2.58	0.36	0.11	0.01	15.16	2.10	0.62	0.09	F
Sample 120094	0.12	0.13	0.00	0.30	0.16	0.00	0.04	0.01	0.00	0.00	3.61	0.56	0.15	0.02	12.05	1.88	0.50	0.08	