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ELEMENTAL ANALYSIS ON SALIVA SAMPLES OF PATIENTS WITH PERI-IMPLANTITIS USING TXRF

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Abstract: The goal of this paper is to examine the composition of saliva samples collected from patients suffering from peri-implantitis and try to find a correlation between the detected elements using TXRF and the disease. We did so for four samples from different regions of the patient's mouth were collected from four subjects. We then added a gallium internal standard to all the samples and let it dry in an oven to perform the measurement using a S2 Picofox from Bruker, then the obtained spectra were analyzed using the Spectra 7 software. By performing the analysis, we noticed that the elements detected on every sample were: Phosphorus, Sulfur, Chlorine, Potassium, Calcium, Iron, Zinc, Bromide and Rubidium. Titanium was also detected on 72% of the samples. Our initial assumption was that the concentration of titanium would be greater in the regions near the compromised implants due to corrosion. We actually found that the titanium concentration was greater at the gingival sulcus of health implants. The results also showed that the concentration of iron and sulfur were greater at the gingival sulcus of compromised implants than anywhere else in the mouth. With these findings, we can confirm that the TXRF is a reliable technique to analyze elements from trace to macro-concentrations and can be used to monitor salivary levels of several elements, but so far, we couldn't find a relation between peri-implantitis and implant corrosion.
Keywords: TXRF, Peri-Implantitis, Saliva.

INTRODUCTION

Mankind has used dental implants to replace missing teeth since immemorial times (SAINI, 2015). As science and technology developed throughout history, so have the materials used to develop new kinds of dental implants. Nowadays, the most common material used to this task is titanium (MOMBELI, 2018). This material is chosen due to its biocompatibility with the oral environment. Titanium implants are surrounded by a layer of titanium oxide TiO_2 that grants protection against corrosion to the implant (SAINI, 2015).

Even though the titanium oxide provide protection to the implants, this layer can be compromised due to some factors such as mechanical wear, pH, bacteria, chemicals and other contaminants (SAFIOTI, 2017; SOLER, 2020). More specifically, bacteria present in the mouth produce toxins that acidify the implant surrounds and can cause dissolution of the titanium oxide layer (MATHEW, 2012). Once the protective layer wears off, the implant becomes exposed to the buccal environment and is now prone to corrosion.

Such disruption of the titanium oxide layer and consequent implant corrosion is what defines the disease known as peri-implantitis (SAFIOTI, 2017). This is an inflammatory disease that affects the peri-implant mucosa and the jaw supporting bone where the implant is installed (LANG, 2011). The diagnostic is given by the BOP (bleeding on probing) exam and radiography of the jaw (SCHWARZ, 2018).

We found papers that chose to analyze the behavior of this disease by employing spectroscopy techniques over tissue samples collect from the gingival mucosa (MERCAN, 2013; HE, 2016) or submucosal plaque (SAFIOTI, 2017). Our aim was to make a similar analysis but using saliva samples instead.

Saliva is a biological fluid whose analysis can provide several information about the subject's health (COLON, 1997). Therefore, performing the elemental analysis on saliva samples collected from individuals with peri-implantitis could provide interesting information about the disease's behavior. 99% of saliva's composition is water, while the remaining 1% is composed by several others organic and inorganic substances (POLETTO, 2021).

Due to the reduced size of the samples and low concentration of elements to analyze, the technique used to perform the analysis was the Total Reflection X-Ray Fluorescence (TXRF). The TXRF is an inexpensive multi-element method suitable for trace-element analysis with a simple sample preparation (VAN GRIEKEN, 2001). This technique was previously applied to saliva analysis by Abraham et al. (ABRAHAM, 2014), Cleto et al. (CLETO, 2016), Borella et al. (BORELLA, 1994), Zahir et al. (ZAHIR, 2006) and Poletto et al. (POLETTO, 2021) among others.

In this study, we hypothesized that the elemental content of samples collected from different regions of different people can reflect the region where each sample was collected, i.e., higher Ti concentrations could indicate that the samples originated from an implant sulcus. The objective of this study was to apply multi-elemental TXRF analysis to the saliva samples and further evaluate them by using Principal Component Analysis (PCA) to check if the samples present some kind of relation. This paper is based on J. Lopes' master's thesis: "Análise Elementar em Amostras de Saliva de Pacientes com Peri-Implantite por TXRF" (LOPES, 2022).

MATERIALS AND METHODS

SAMPLE COLLECTION

Four samples were collected from different mouth regions from four subjects. The relation between patients and region from where the samples were collected can be seen on Table 1. All patients agreed to take part on this study and the samples were collected during routine dental exams.

TXRF SPECTROMETER

The device used to perform the measurements was the S2 Picofox (Bruker Corporation, Berlin, Germany), located in the Applied Nuclear Physics Laboratory at the Londrina State University, Parana State, Brazil. The system is a transportable TXRF spectrometer that dispenses cryogenic refrigeration, therefore is useful not only in a laboratory but also for on-site analyses in the field. The device performs simultaneous and fast determination of all elements from sodium to uranium and can detect concentration limits as small as ppb.

This spectrometer is specially indicated for environmental analysis, quality control. material science, medicine and biotechnology. The voltage, the current and the maximum power that can be applied to the molybdenum

Region	**Patient**			
	1	**2**	**3**	**4**
Parotid gland duct	X	X	X	X
Healthy implant sulcus	X	X		X
Implant interior	X			X
Peri-implantitis sulcus	X	X	X	X
Total saliva		X	X	
Peri-implant mucositis			X	

Table 1: Relation between patients and samples.

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X-ray tube built within the device are 50 kV, 600 μA and 30 W, respectively.

The Picofox system is designed for sample carriers that are 3 cm of diameter and 3 mm in height. Among the types of samples carriers indicated for the device, we chose to use acrylic discs since they are cheap, disposable and presents a small blank on each measurement.

Once the saliva was deposited on the discs, 10 μL of an internal standard of gallium at the concentration of 5 mg/L were pipetted over each sample. After that, the samples were taken to an oven at 55°C for approximately 35 minutes to dry out and then be measured. Each sample was measured three times for a live time of 100 seconds. The precision detection varied for each element on each measurement and is reported as the lower limit of detection (LLD).

QUANTIFICATION AND DATA ANALYSIS

Quantification of the samples was performed with respect to the internal standard using the Spectra 7 software, develop specifically to use alongside the S2 Picofox system. To validate the built-in calibration of the software we used two reference material: NIST 1640 and NIST 1643e. Mean concentrations and standard deviations were calculated with MatLab software and bar graphs comparing the concentrations of each element detected on the samples were plotted using Microsoft Excel. Principal Component Analysis (PCA) was also performed using MatLab.

Due to the high divergence encountered for concentration values along the samples, we opted to carry out the PCA using the z-scores of each value instead of the actual concentrations. The equation used to find these scores is written below,

$$Z = \frac{|X - \bar{X}|}{\sigma}$$

where Z represents the standardized concentration, X is the concentration to be standardized, $\bar{X}$ is the mean concentration for a given element across all samples and σ is the standard deviation for this distribution. The module had to be taken since the PCA requires positive integers.

RESULTS AND DISCUSSION

The elemental concentrations detected by the Picofox system were determined by comparison between the detected counts for each element peak in comparison with measured for the internal standard. A typical spectrum is shown in Figure 1.

Along the measurements, the detected elements were: Al, Si, **P**, **S**, **Cl**, **K**, **Ca**, Ti, V, Cr, Mn, **Fe**, Co, Ni, Cu, **Zn**, As, Se, **Br**, **Rb** and Sr. Those written in bold were detected on all sixteen samples. Titanium wasn't detected on any sample from one of the patients but was detected on every other sample. Therefore, we decided to consider its behavior in our analysis.

The concentrations measured for each element on each sample are displayed on Table 2 alongside its respective z-scores. These concentration results are also displayed on the bar plots from Figures 2 through 5.

By examining the table, one can notice that the concentrations values encountered diverge very much from one another, therefore the standardization is necessary to proper analysis the elements behavior. Beyond that, the bar charts were plotted displaying two scales to aid on the visualization.

There are three immediate results that can be taken from the bar plots:

- The Parotid gland samples presents the lowest concentration of elements for all four subjects;

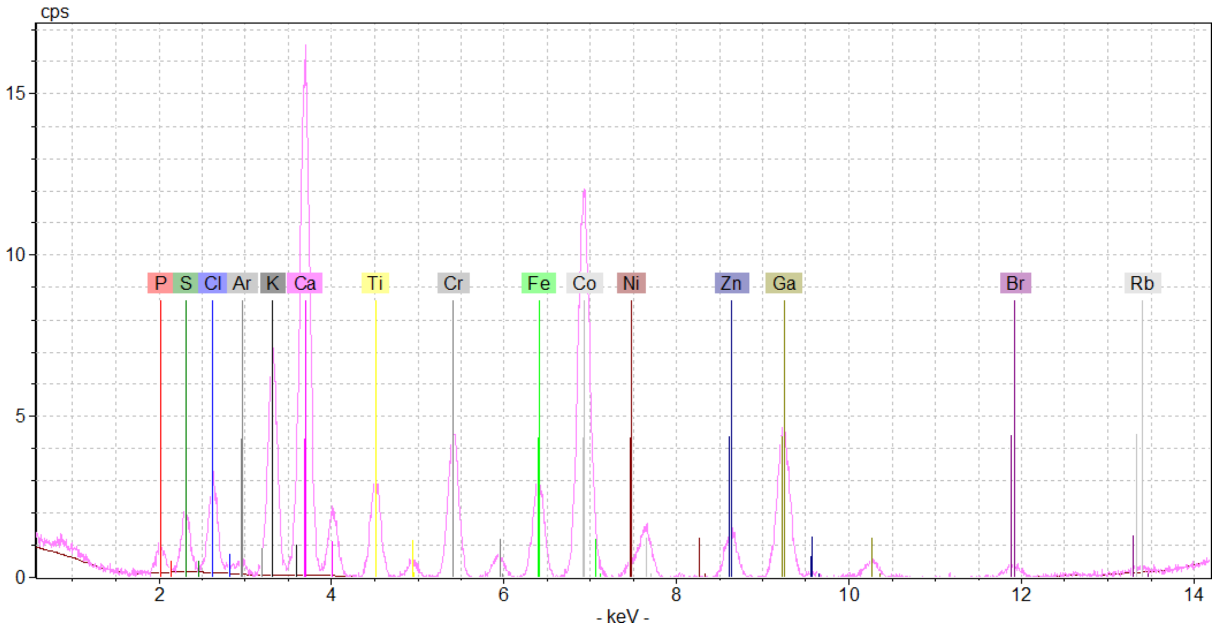

Figure 1: A typical TXRF spectrum for saliva samples.

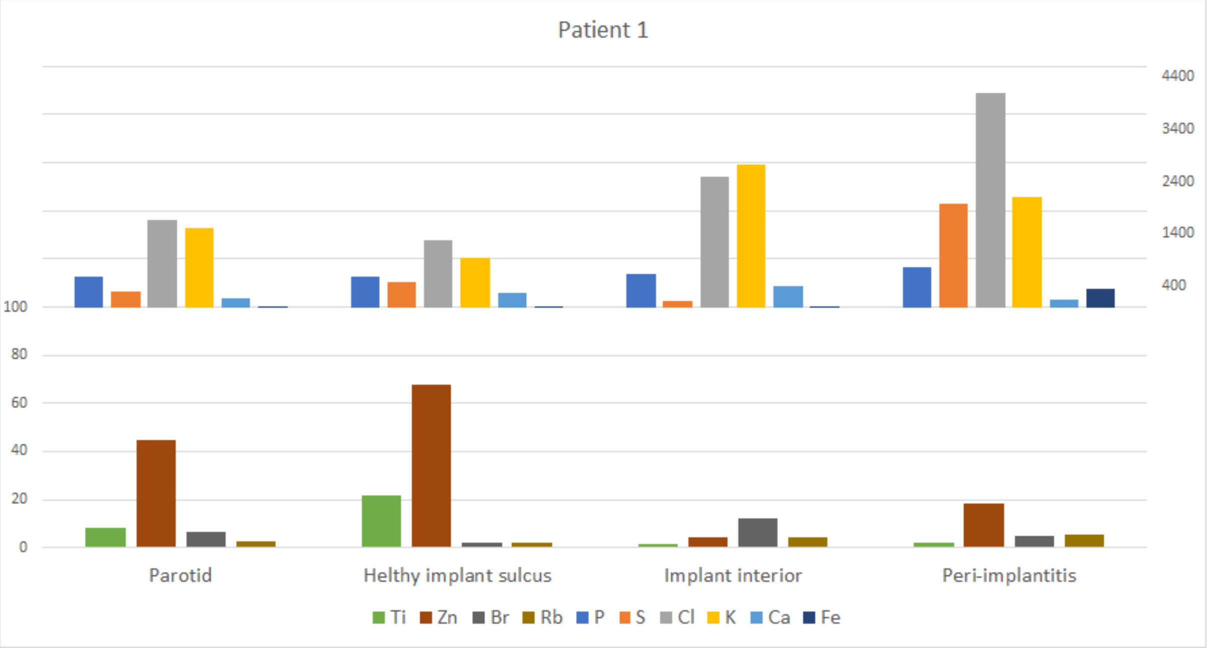

Figure 2: Measured concentrations for the first patient.

			P	S	Cl	K	Ca	Ti	Fe	Zn	Br	Rb
Patient 1	Parotid	C	571,777	296,769	1657,553	1522,850	179,470	8,075	0,821	44,865	6,627	2,792
		Z	0,314	0,172	0,381	0,167	0,062	0,428	0,326	1,792	0,265	0,344
	Healthy implant sulcus	C	575,615	488,519	1272,071	948,546	276,633	21,782	7,176	67,643	2,118	1,943
		Z	0,325	0,177	0,046	0,325	0,712	2,179	0,253	2,990	0,668	0,114
	Implant interior	C	644,031	127,666	2494,580	2715,303	399,286	1,748	9,935	4,428	12,348	4,576
		Z	0,515	0,479	1,107	1,190	1,532	0,380	0,221	0,336	1,450	1,306
	Peri-implantitis sulcus	C	766,180	1980,317	4105,117	2102,243	151,983	2,353	347,553	18,173	4,897	5,480
		Z	0,855	2,886	2,505	0,664	0,122	0,303	3,659	0,387	0,093	1,793
Patient 2	Parotid	C	276,844	16,208	56,593	873,042	66,253	0,153	0,215	0,624	4,456	1,003
		Z	0,507	0,681	1,009	0,390	0,695	0,584	0,333	0,536	0,184	0,621
	Peri-implantitis sulcus	C	1316,917	1444,686	2165,709	3651,269	314,938	1,028	4,416	16,511	12,816	3,630
		Z	2,388	1,913	0,822	1,993	0,968	0,472	0,285	0,300	1,547	0,796
	Healthy implant sulcus	C	799,671	387,188	1920,310	2240,240	210,808	0,930	2,796	3,926	10,311	2,944
		Z	0,948	0,008	0,609	0,783	0,272	0,485	0,303	0,362	1,028	0,426
	Total saliva	C	608,426	171,543	2038,566	2978,192	535,077	0,940	2,683	6,113	10,775	4,886
		Z	0,416	0,399	0,712	1,416	2,440	0,484	0,304	0,247	1,124	1,473
Patient 3	Peri-implantitis sulcus	C	91,797	159,053	219,692	159,300	9,740	0,000	0,693	0,896	0,463	0,390
		Z	1,022	0,422	0,867	1,002	1,073	0,604	0,327	0,522	1,011	0,951
	Peri-implant mucositis	C	27,036	14,705	4,363	49,371	5,948	0,000	0,000	0,314	0,142	0,026
		Z	1,202	0,684	1,054	1,096	1,098	0,604	0,335	0,552	1,077	1,148
	Total saliva	C	299,723	120,379	345,758	873,741	56,053	0,000	0,347	0,815	6,117	1,021
		Z	0,443	0,492	0,758	0,389	0,763	0,604	0,331	0,526	0,160	0,611
	Parotid	C	3,880	1,149	1,783	1,198	1,983	0,000	0,000	0,133	0,054	0,009
		Z	1,266	0,709	1,056	1,138	1,125	0,604	0,335	0,562	1,096	1,157
Patient 4	Peri-implantitis sulcus	C	245,275	515,837	1019,345	551,991	90,820	1,310	78,213	2,642	1,151	1,128
		Z	0,595	0,226	0,173	0,665	0,531	0,436	0,564	0,430	0,868	0,553
	Implant interior	C	71,373	62,044	72,100	74,878	120,872	14,895	6,444	1,773	0,339	0,123
		Z	1,078	0,598	0,995	1,074	0,330	1,299	0,261	0,476	1,037	1,095
	Healthy implant sulcus	C	280,696	382,042	725,989	421,222	175,575	22,395	4,854	3,414	1,296	0,859
		Z	0,496	0,017	0,428	0,777	0,036	2,258	0,279	0,389	0,838	0,698
	Parotid	C	763,201	93,258	1400,752	2079,071	127,106	0,000	0,596	0,709	11,611	3,657
		Z	0,847	0,541	0,158	0,644	0,288	0,604	0,328	0,532	1,297	0,810

Table 2:Concentrations and respective z-scores for each measurement.

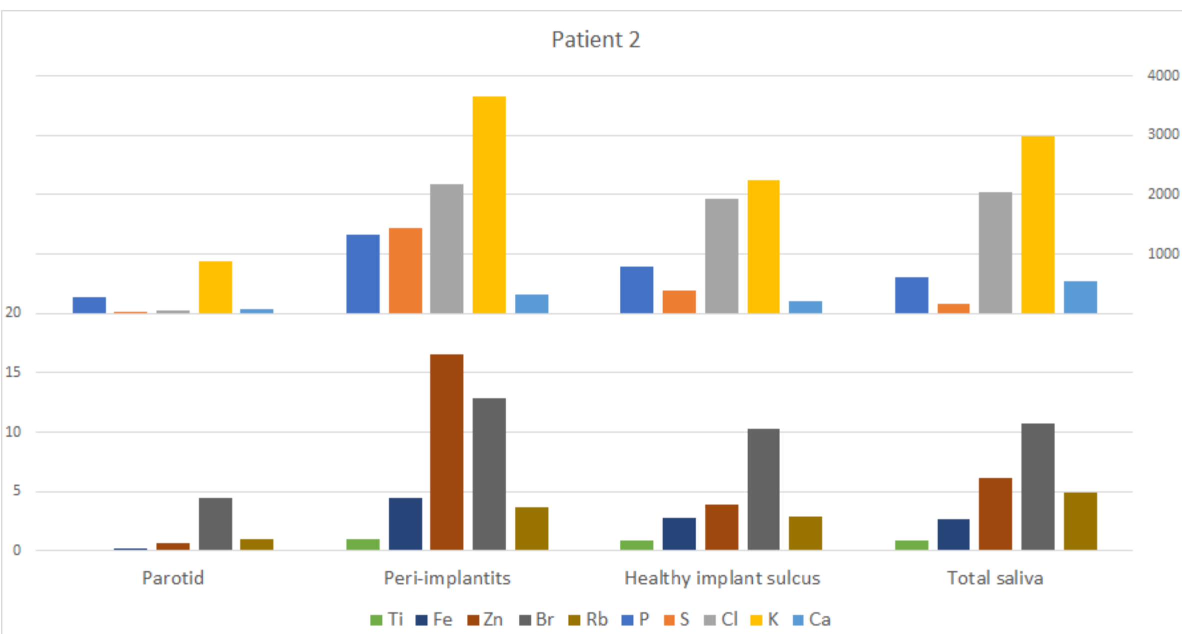

Figure 3: Measured concentrations for the second patient.

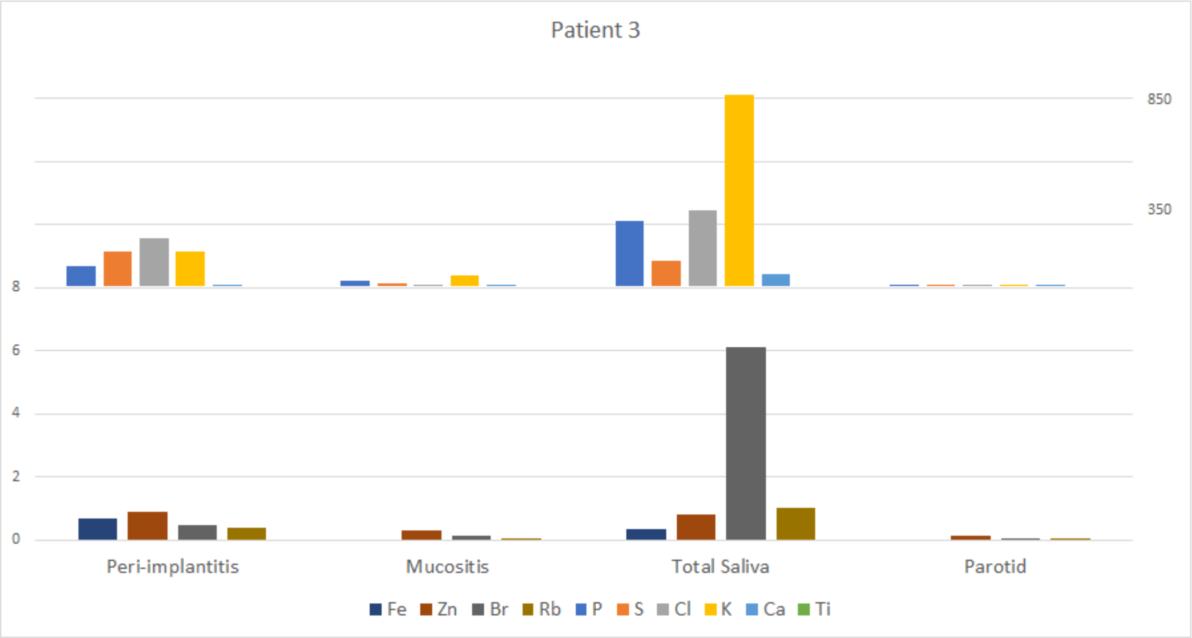

Figure 4: Measured concentrations for the third patient.

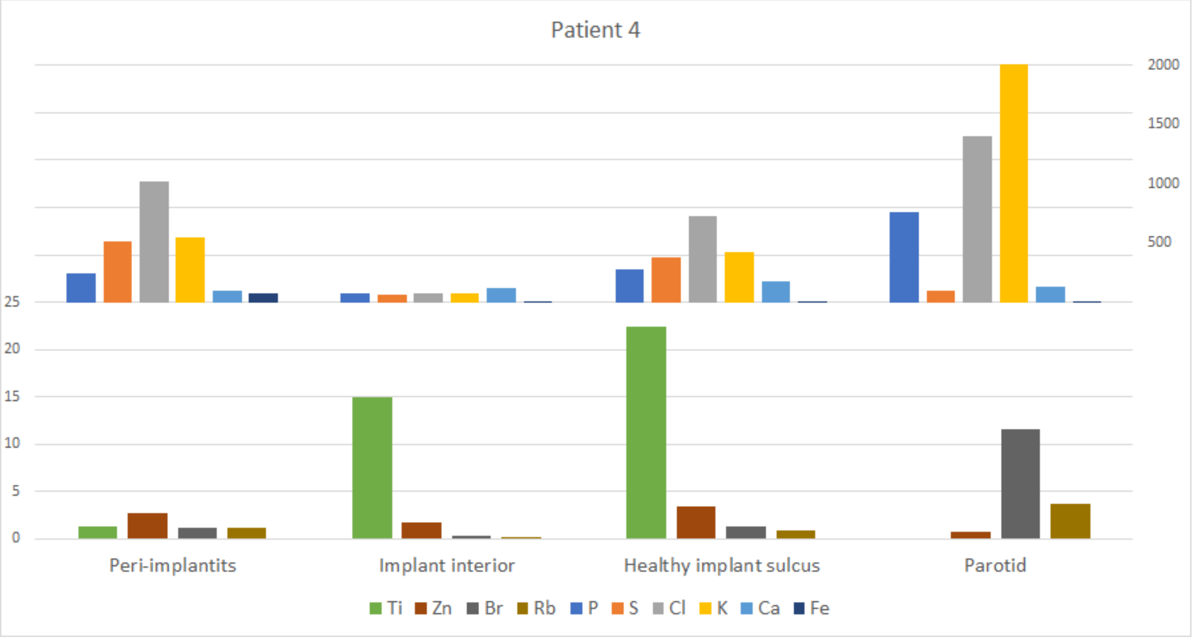

Figure 5: Measured concentrations for the fourth patient.

- The sulfur and iron concentration are higher on peri-implantitis sulcus than any other regions; and
- The titanium concentration is higher on healthy implant sulcus than on peri-implantitis sulcus.

The fact that the third result goes against what was found by Mercan et al. (MERCAN, 2013) suggests that the titanium corroded from implants is deposited in the gum instead of being diluted on saliva.

Using the values obtained by calculating the z-scores we also performed the PCA for all samples, the result is shown on Figure 6. Unfortunately, no discerning behavior was obtained, it is not possible to infer the regions from which the samples were taken because there are no cluster formations.

Therefore, even though the TXRF is a reliable technique for elemental composition and saliva analysis, we can't seem to find a relationship between a sample composition and its origin by using chemometric analysis (PCA).

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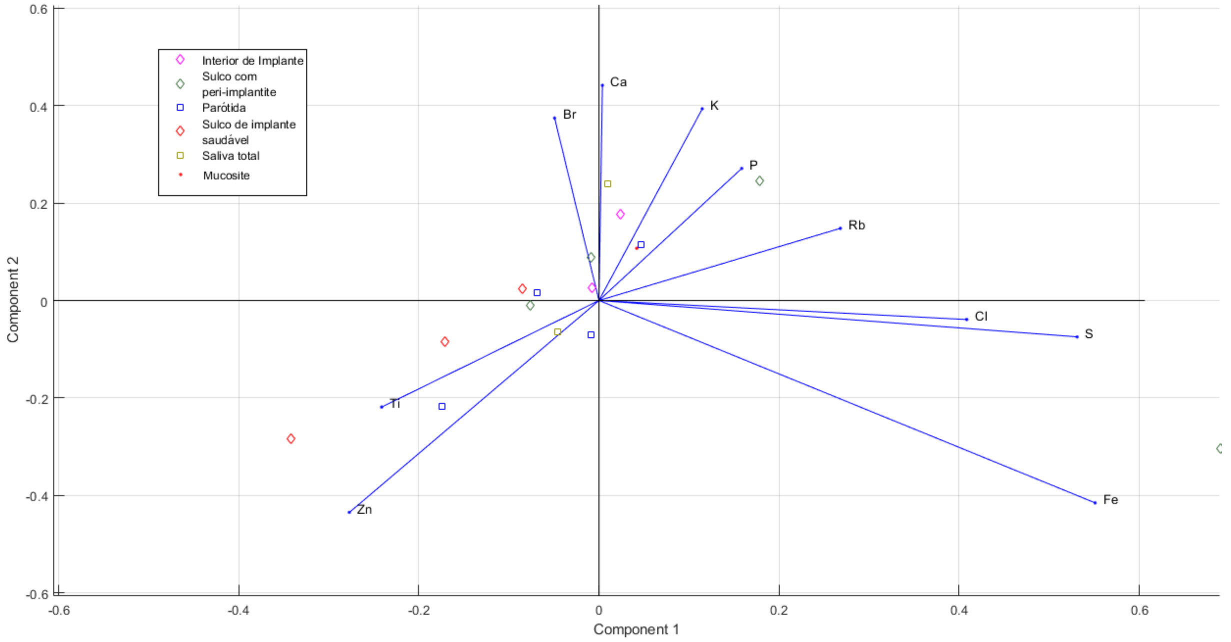

Figure 6: PCA for the standardized results.

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