ICP-MS IN EVALUATION OF HEAVY METAL CONTENT IN ORAL LICHENOID

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Oral lichen planus and oral lichenoid lesions have similar clinical features and carry an important risk of malignant transformation. While oral lichen planus is an idiopathic disease, oral lichenoid lesions are caused by dental materials (alloys), drugs or morrow grafts. The aim of this study is to find out if there are any differences between heavy metals concentrations in oral mucosa with lichenoid lesions and a control group. The trace metals were analyses was performed using inductively coupled plasma mass spectrometry (ICP-MS) method.

Keywords: heavy metals, oral lichenoid lesions, dental crowns, dental alloys, inductively coupled plasma mass spectrometry, energy dispersive X-ray analysis

1. Introduction

Lichen planus is a common chronic inflammatory autoimmune disease, T-cell-mediated, with unclear aetiology which may affect both, skin and oral mucosa [1,2,3]. Oral lichen planus affects stratified squamous epithelia from the oral cavity, especially from bucal mucosa, tongue, and gingiva, lesions that can be found bilateral almost every time [4]. In contrast with oral lichen planus, oral lichenoid lesions (OLL) have a unilateral tendency and they are more often found very close to a dental restoration (crown or direct amalgam restoration) [2,4].

There are few studies which found trace of metallic elements in OLL [4-6], which can be a possible cause or trigger element for these lesions. Moreover, the OLL seem to be in regression when metallic dental crowns or bridges are removed and replaced [2]. However, even the studies mentioned above did not use

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the inductively coupled plasma mass spectrometry (ICP-MS) as method for detection trace metals in oral tissues, this method was previously used for finding any ions released from fixed orthodontic appliances [7,8], dental implants [9] and temporary teeth [10,11]. This method is an analytical technique capable of finding trace metals at concentration as low as one part per quadrillion (ppq) combining a high-temperature inductively coupled plasma (ICP) source with a mass spectrometer to separate and quantify metallic ions [12].

The main purpose of this study is to compare the level of heavy metals in oral mucosa of patients who have metallic dental restorations with or without OLL, using ISP-MS method.

The data presented in this paper were obtained as part of an in vivo study conducted from February to September 2016 in both, Department of General Chemistry, Faculty of Applied Chemistry and Materials Science, Politehnica University of Bucharest and Department of Oral Pathology from the School of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest.

2. Materials and Methods

2.1. Study design and sampling

Eight patients were selected for this study, five of them suffering from OLL (Fig. 1 and 2). All these lesions were detected through oral examination in contact with a dental crown or bridge, most of them being keratosis of oral mucosa. The tissue samples were extracted from these lesions or healthy oral mucosa using standard core biopsy and collected then into sterile plastic containers. A part of tissue samples were analysed using hematoxilin-eosin stain and the OLL diagnosis was confirmed by histopathology.

A questionnaire was also used to exclude other sources of metallic ions but dental crowns: metabolic diseases, drugs, vitamins or minerals ingestion, and diet.

The rest of three patients were kept for control.


Fig. 1: OLL on bucal mucosa


Fig. 2: OLL on alveolar mucosa under a, dental bridge; the restoration was removed

2.2. Tissue samples analysis

The concentration of metallic ions in oral tissue samples was determined using inductively coupled plasma mass spectrometry (ICP-MS). It was used type ELAN DRC-e Perkin Elmer SCIEX U.S.A. with a detection limit as 0.001 μg g. Calibration function for the ICP-MS spectrometer was generated using a multi-element standard. All samples were digested in 100 ml concentrated nitric acid (ULTRAPURE, Fa. Merck). Acid digestion was done in a well determined volume of HNO3 65%; after digestion, the samples were diluted 100 times and liquid fractions were analysed.

2.3 Dental crowns' analyses

Some of crowns and bridges in contact with OLL were removed and analysed using a Hitachi SU8230 microscope equipped with a EDAX mode at a voltage of 10 kV. Energy Dispersive X-ray (EDX) System was used for different segments of dental crowns.

3. Results and Discussion

The trace metallic elements from the oral mucosa samples had the following values:

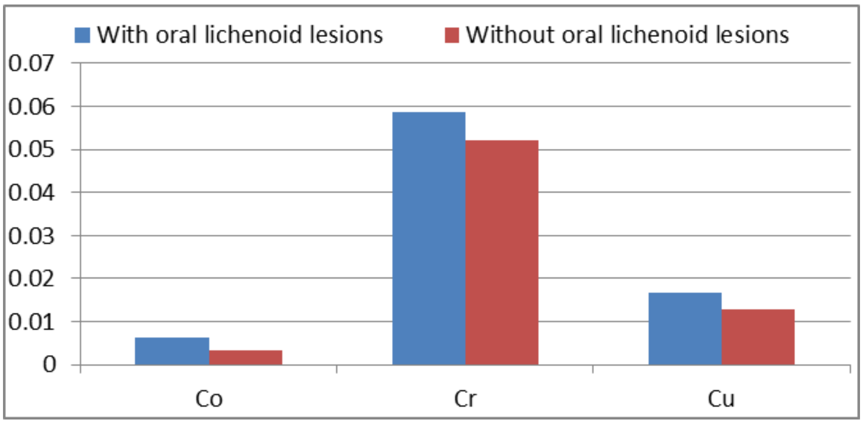
Table 1

Content of heavy metals in oral mucosa samples in ppm (mg/L)

Patient	OLL	Co	Cr	Ni	Mo	Fe	Cu
1	Yes	0.003	0.046	112.739	9913.851	1.246	0.025
2	Yes	0.004	0.070	117.094	9811.198	2.107	0.013
3	Yes	0.003	0.061	104.808	9631.127	1.781	0.015
4	Yes	0.009	0.058	56.972	9933.611	2.211	0.015
5	Yes	0.013	0.058	76.031	6539.880	1.595	0.015
6	No	0.003	0.056	110.642	9874.153	1.326	0.015
7	No	0.004	0.043	69.875	7894.198	1.254	0.016
8	No	0.003	0.058	69.875	9811.198	1.306	0.008

The patients were divided in two groups: with and without OLL. Medium values for each ion were calculated and the results were graphically exposed below (figures no. 3 and 4:a & b).

As can be seen below, almost all metallic ions have high concentrations in mucosa samples of the OLL than in healthy oral mucosa. However, it is very difficult to figure out which is the true source for trace metals in oral mucosa. Some elements such as Zn and Cu are essential for human body [4], this being the main reason for selecting our patients and exclude some of them based on a questionnaire about their diet, diseases, and medication.



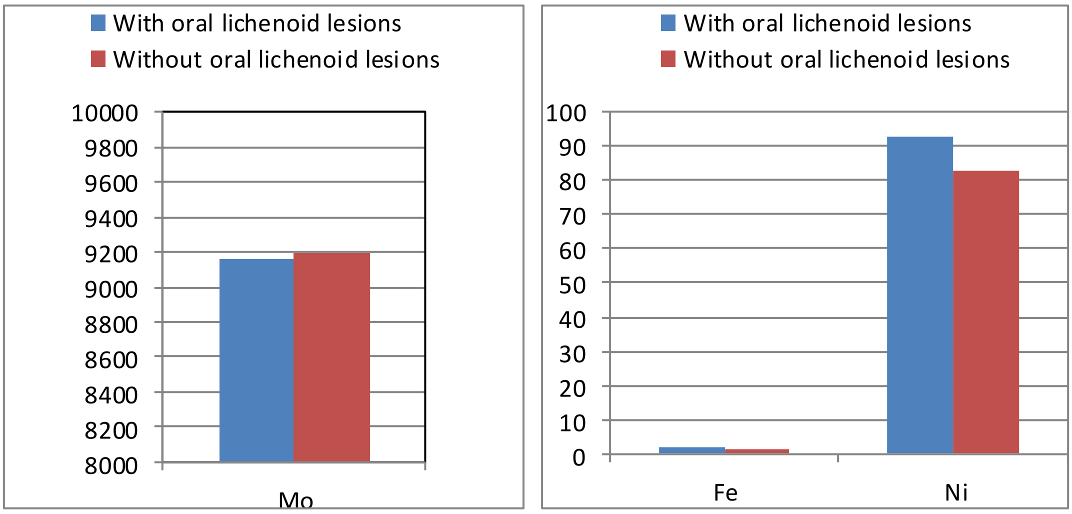


Fig. 3: Co, Cr, and Cu ions in oral mucosa samples

Fig. 4 (a: left & b: right): Fe, Ni, and Mo ions in oral mucosa samples

Bonda et al. found increased concentrations of Cr, Co, and Ni analysing oral mucosa samples from patients suffering from OLL and wearing metallic dental crows compared to a control group [5]. We also found higher percentages of Ni, Fe, Cr, Cu, and Co in the mucosa samples of OLL patients compared to patients with normal mucosa. OLL are strong related to allergy to dental materials. The dental alloys are also elements which can lead to positive reactions of patch tests. Ditrichova et al. found that nickel and cobalt, elements which also been discovered in this study, are some of the most involved in these reactions [13].

In Romania, three main types of alloys were used for dental crowns and dentures: Ni & Cr, Cr & Co, and Cu & Al (Gaudent). However, the new alloys that have recently started to be used in Romania have no Ni but high concentrations of Cr and Mo for better mechanical properties and high resistance to corrosion [14].The Gaudent alloy which is based on cupper has begun to be used increasingly less in dentistry in Romania. Some studies proved that cupper-based dental alloys can induce cytotoxic effects interfering with lymphocytes viability and proliferation. However, it is well known that cupper is essential for life, but being a component of different dental alloys it can induce carcinogenic activity of other ions, such as Ni [15].

Trying to find out what are the main components of alloys that dental crowns are made from in our OLL group, we removed and analysed some of them using EDX System. The results showed from the beginning a high degree of physical wear, and a lot of craters, crevices and pitting on their external surfaces. The crowns also have a heterogeneous morphology and a composition which varies very much, as was confirmed by the EDAX measurements. In the figures no. 5 – 12 are shown EDAX analyses for two types of dental crowns: made from Ni & Cr and Cu alloys (Gaudent).The percentages of Ni and Cu ions are the highest.

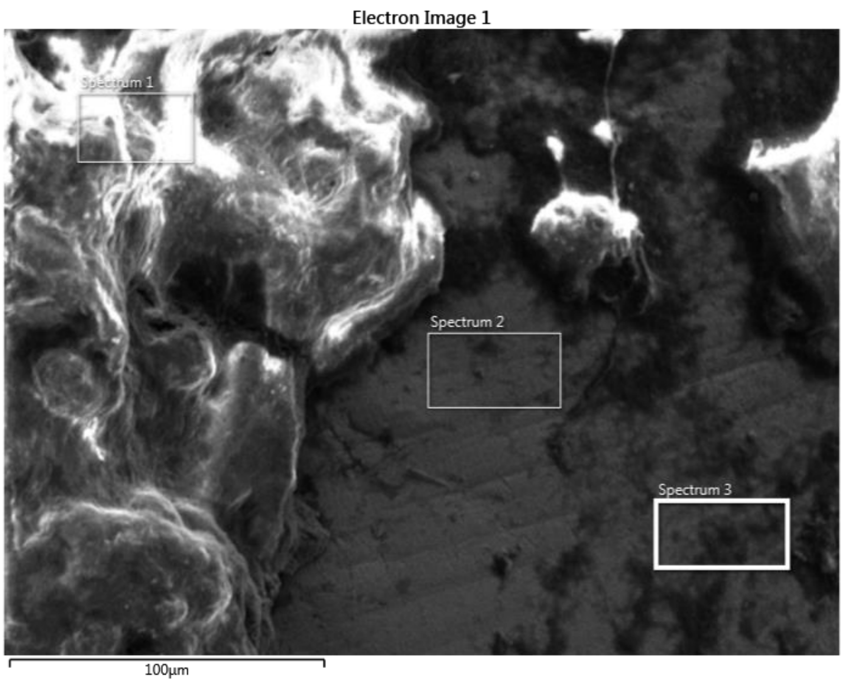


Fig. 5: An electron image of a semi-physiognomic metal crown (Ni & Cr alloy)

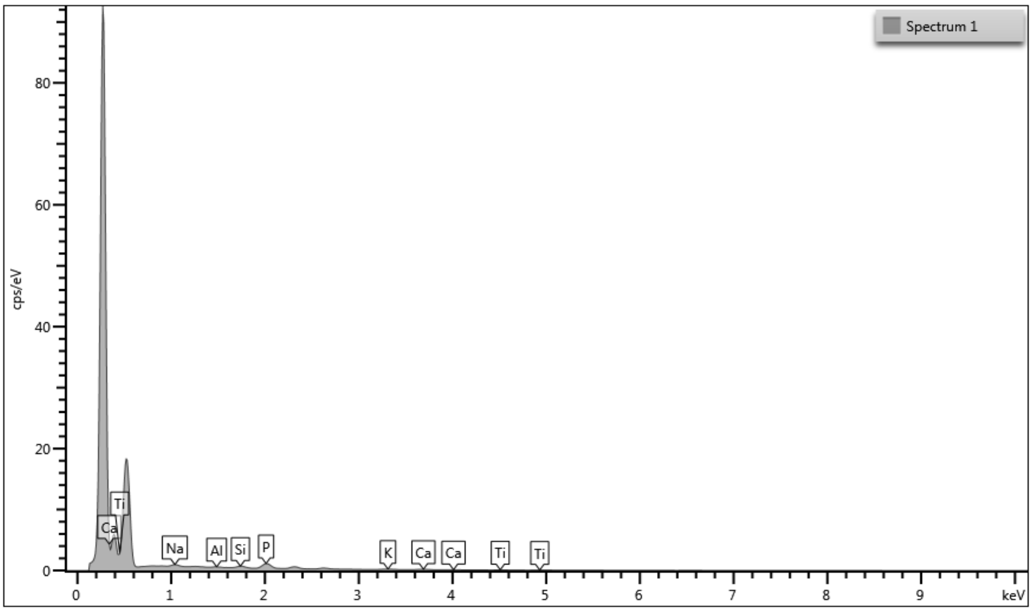


Fig 6: EDAX measurements for spectrum 1

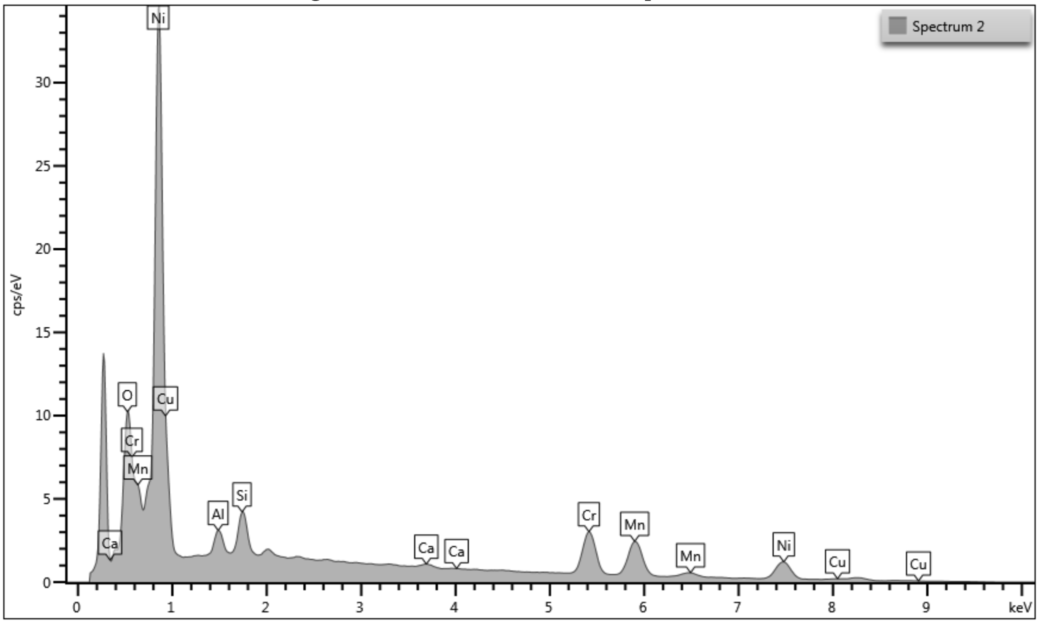


Fig 7: EDAX measurements for spectrum 2

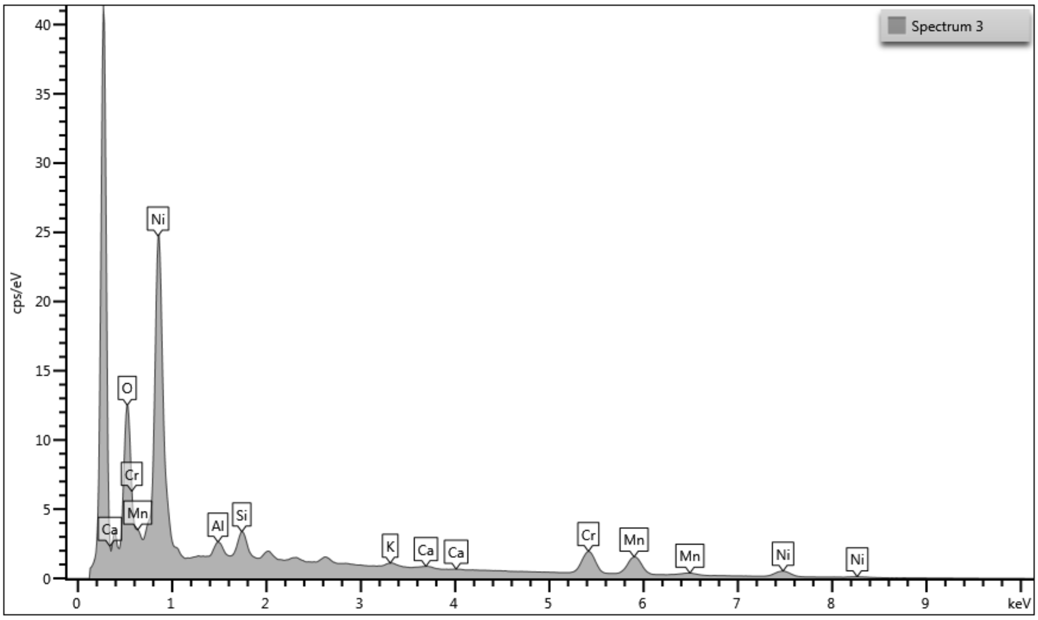


Fig 8: EDAX measurements for spectrum 3

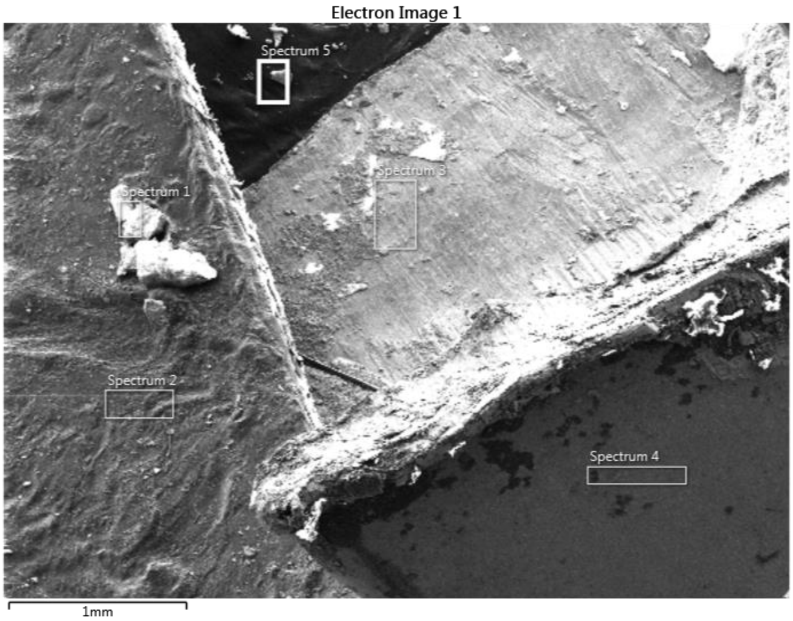


Fig. 9: An electron image of a metal crown made from Gaudent (Al & Cu alloy)

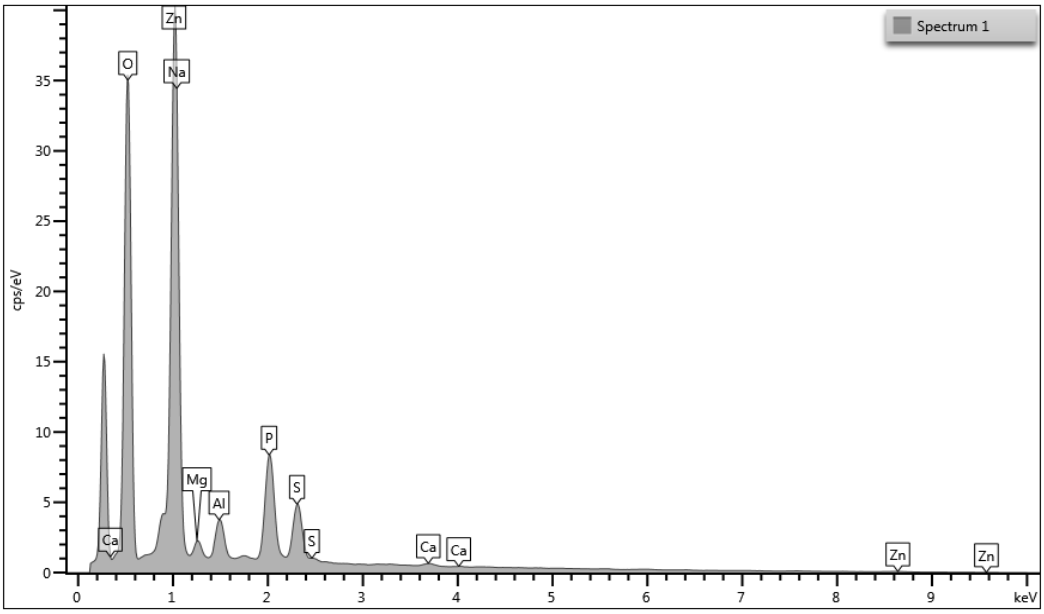


Fig. 10: EDAX measurements for spectrum 1

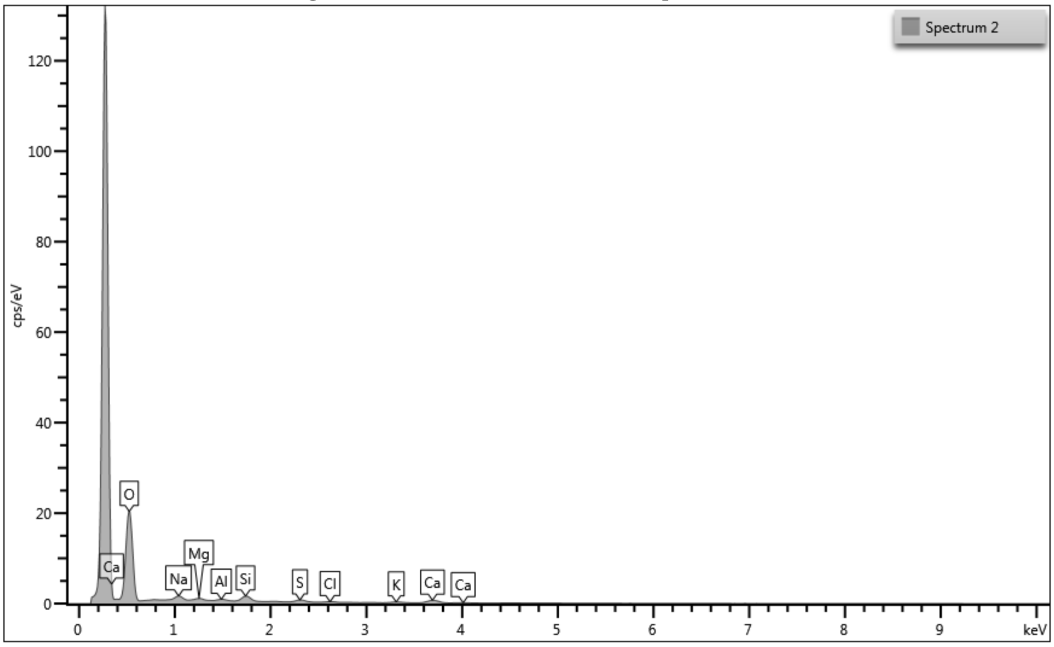


Fig. 11: EDAX measurements for spectrum 2

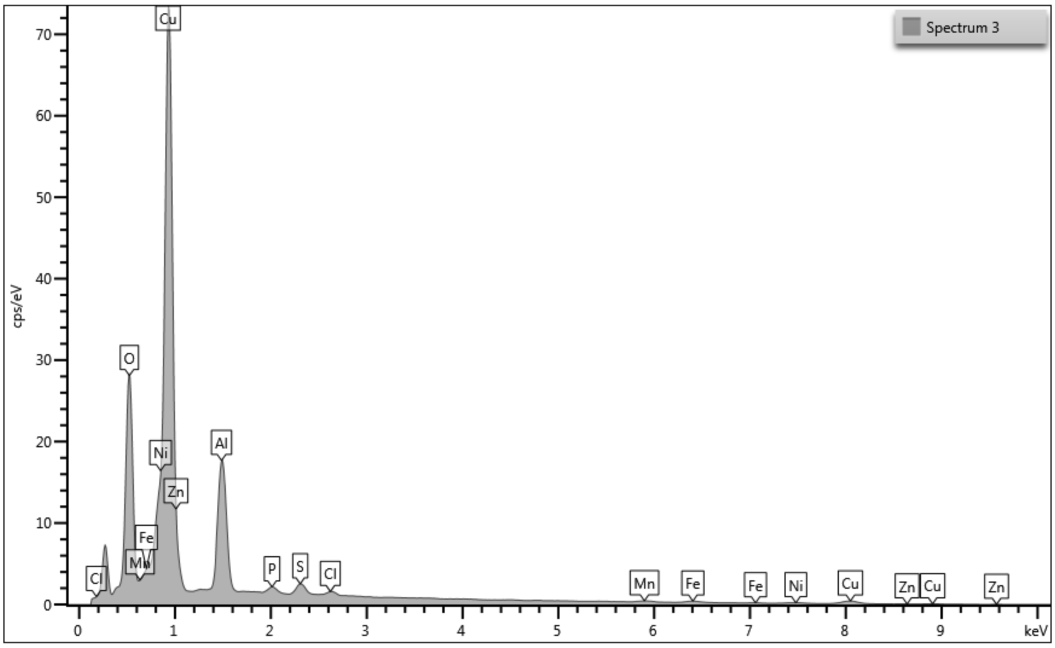


Fig. 12: EDAX measurements for spectrum 3

This study has also some limitations. Since the number of patients from both, control and OLL groups was low and the histopathology was performed only for OLL diagnosis confirmation (not for trace metallic elements identification), we cannot say that it is a direct link between ions released from dental alloys and OLL. Moreover, the core biopsy was performed using metals instruments, which may lead to some false positive results.

4. Conclusions

Our study shows that some trace metals have unusual high concentrations in OLL mucosa samples. It is likely that crowns or bridges may be the main source for these metallic traces since the diet and other ions sources were expelled from the beginning. Even the alimentary source and medications were excluded, the low number of patients requires further investigation in the future in order to establish whether exists a direct link between trace metallic elements from oral mucosa and OLL.

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