

## ANALYSIS OF MASS PORTIONS OF BIOGENIC ELEMENTS IN DENTAL TISSUE

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**Abstract:** Introduction: Hard dental tissues represent ideal tissues for assessing the long-term effects of exposure to toxic metals. The aim of this paper was to determine the representation and the relation between mass portions of the following elements: carbon, oxygen, sodium, magnesium, aluminum, chlorine, potassium, iodine and lead in certain parts of the teeth (enamel-dentine line, dentine, pulp) with rats with experimentally induced diabetes mellitus (DM) by using SEM/EDS analysis, after 14 and 30 days of exposing animals to lead.

Material and methods of work: The study was conducted in rats of Wistar strains divided into two groups. The first experimental group (A1) consisted of 8 rats, taking lead in the course of 14 days at a concentration of 1500 ppm and the second experimental group (A2) consisted of 8 rats taking lead in the course of 30 days at a concentration of 1500 ppm. The rats from group A1 and A2 had induced diabetes mellitus by using the Alloxan which was administered intraperitoneally at a dose of 100mg per kilogram of body weight. The teeth samples were analyzed by scanning electron microscopy (SEM).

Results: No lead was detected in the teeth of rats with experimentally induced diabetes that received lead in drinking water in the course of 14 days, while the average values of mass portions of other examined elements amounted to: carbon -24,25 %, oxygen - 38,17%, sodium -0,9%, magnesium -0,11%, aluminum - 0,07%, chlorine - 0,21% and iodine - 0,32%. The average values of the mass portions of examined elements in the enamel of teeth of rats receiving lead in the course of 30 days amounted to: lead -0,36%, carbon-31,09%, oxygen - 41,13%, sodium - 0,91%, magnesium - 0,21%, chlorine - 0,22%, potassium - 0,03% and iodine - 0,17%.

Conclusion: Mass portions of elements found in the teeth enamel of rats receiving lead in the course of 30 days with experimentally induced diabetes were higher but with no statistically significant difference compared to the mass portions of elements in the group of teeth of rats taking lead through drinking water in the course of 14 days with experimentally induced diabetes, too. The lead was detected in the teeth of rats that received lead for 30 days with induced diabetes but only in the enamel.

**Keywords:** mass portions of elements in the enamel, dentinogenesis, SEM/EDS analysis.

### 1. INTRODUCTION

In the constituent sense, all living beings are made of both organic matter and chemical elements (macroelements, microelements) and from toxic elements that are found in different concentrations in the body due to environmental contamination (Al, Hg, Cd, Pb, Bi, Ag). Among the toxic elements, lead is especially distinguished as one of the most important and the most toxic heavy metals due to the fact that the environment is becoming more and

more polluted [1,2]. Polluting the environment with heavy metals leads to the fact that the human body absorbs them and deposits in the liver, bones and teeth. This is why the teeth are suitable biomarkers for determining exposure to metals, especially lead [3]. The relationship between lead and the metabolism of bioelements was examined with particular care during researches. The deposited lead in solid tissues, according to many authors, does not necessarily represent a danger to the organism, nor must manifest toxic effects in them except in cases

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of sudden mobilization and transition to blood due to certain physiological or pathological processes. Enamel as the most mineralized tissue in the organism makes a protective cover of the pulpo-dentine complex and in addition to hydroxyapatite its inorganic composition (96–97%) consists of small concentrations of magnesium, carbonate and fluoride too, whose composition and structure are changed under the influence of various etiological factors and heavy metals from the environment [4,5]. Reactive dentinogenesis occurs in mild and moderate stimuli where odontoblasts can survive and enhance their secretory activity by forming new layers of tertiary dentine [5]. On the basis of the SEM examination of pathological dentinogenesis, Vojinovic et al. concluded that the structure of the pathological dentine is very similar to the structure of the cover dentine and that it depends on the severity and length of the irritation [6]. More recently, Tvinnereim et al. have noticed, through the study, a connection between lead exposure at the time of dentine formation and a 40% increase in sensitivity to caries in rat teeth [7].

In some studies, the effect of lead on delayed tooth growth in some areas of heavy metals contamination was confirmed [2,8]. There are reports in the literature indicating that the presence of lead in the chemical composition of the enamel can change its dental ultrastructure and lead to damage of the enamel. Thus, Gomes et al. found that the teeth of pre-school children living in the industrial area of the city had a higher concentration of lead in the enamel than the teeth of children living in the non-industrial area [9]. Teeth with increased lead content also show increased abrasion and discoloration because the porous structure of the enamel is more sensitive to abrasion and absorption of exogenous pigments. However, these studies were not sufficient to establish a clear correlation between the presence of lead and such damages of enamel [10,11]. It has also been confirmed that intense metabolism of the pulp is significantly slowed down in some metabolic disorders, such as diabetes, and therefore additional researches of lead deposit under these conditions are necessary [12].

Diabetes mellitus (DM) is a chronic disease that negatively affects the pulp reparation (reparative ability of the pulp) as well as the periodontium, as it has been confirmed by numerous studies. DM represents a predisposing factor to caries, gingivitis, periodontitis, oral candidiasis and xerostomia, and many other oral cavity diseases [13,14]. Microcirculation of the pulp is also an important dynamic system that regulates the metabolic processes of the tooth and dentinogenesis. The blood

vessels of the pulp are influenced by numerous neurogenetic factors as well as by the physico-chemical factors that can influence the defense reactions of the pulpo-dentine complex. Enamel hypoplasia is an important clinical problem in neonates whose mothers have had diabetes. [15]. Silva et al. examined whether there is enamel hypoplasia in the teeth of rats (infants) of the Wistar strain, where females were brought into diabetes during pregnancy. White defects in the enamel (hypoplasia) were observed microscopically which was confirmed by SEM analysis, where hypoplasia of the enamel was recorded in almost all teeth of the experimental group [10]. The parameters of the antioxidant system have been significantly altered in the pulp of the rats with experimentally induced diabetes, which was confirmed by one of the studies [15]. While mechanisms of parodontium disease in DM are completely clarified, cellular and molecular mechanisms of dental pulp disorders during diabetes remain a problem that is being investigated [16]. Histopathological studies show the existence of angiopathy and thickening of the basal membrane of both small and large blood vessels of the pulp [17]. It has also been confirmed that intense pulp metabolism is significantly slowed down in some metabolic disorders (such as diabetes) and therefore additional studies of lead deposit in these conditions are necessary. Reparative dentinogenesis implies the formation of tertiary dentine in response to appropriate pathological irritations (exogenous or endogenous). The production of dentine, which has been increased during reparation (tertiary dentinogenesis) is characteristic only for pulp tissue. The newly formed dentine is deposited on the pulpodentine line, in order to protect the pulp against the effects of harmful irritations [18].

The aim of this study was to determine the representation and interaction of the mass fraction of the following elements: carbon, oxygen, sodium, magnesium, aluminum, phosphorus, chlorine, potassium, iodine and lead in certain parts of the tooth (enamel, enamel-dentine line, dentine and pulp) of rats with experimentally-induced diabetes mellitus after 14 and 30 days of exposure to lead, by using SEM-EDS analysis.

## 2. MATERIAL AND METHOD OF WORK

Wistar strain rats were selected as the sample, due to the great similarity in the physiology of the tooth pulp of the rats with the physiology of the human tooth pulp. The experiment included 16 laboratory rats of Wistar strain, or 336 teeth. The

study was approved by the Ethics Committee of the Institute of Dentistry of the Faculty of Medicine in Banja Luka. Experimental groups of rats were divided into two groups: the first (A1) group consisted of 8 rats (128 molars and premolars of the upper and lower jaws) taking lead in the course of 14 days at a concentration of 1500 ppm ad libitum. The second experimental group consisted of 8 rats (128 molars and premolars of the upper and lower jaws) taking lead in the course of 30 days at a concentration of 1500 ppm, also ad libitum. The rats from group A1 and A2 had induced diabetes mellitus by using the Alloxan which was administered intraperitoneally at a dose of 100mg per kilogram of body weight. All animal procedures were conducted in accordance with the Guidelines for care of animals used for experimental researches („Guide for the Care and Use of Laboratory Animals“, 1996 National Academy Press, Washington, DC). Animals were sacrificed by decapitation under deep etheral anesthesia after the predicted period of lead intoxication. The upper jaw bones of the rats were separated from the soft tissues and stored in fixative (10% neutral buffered Formalin). The teeth samples were analyzed by scanning electron microscopy (SEM) At this stage of the experiment, the material was sent to the University Centre for Scanning Electron Microscopy in Novi Sad, where material preparation for the SEM-EDS analysis was made. The tooth samples were cut and polished with a diamond disc through the middle of the tooth in the medio-distal direction in order to expose the cross-section of the enamel zone and dentine mass by using Ion Sputter Coating Bal-Tec SCD 005. Samples were dried and prepared for SEM analysis, steamed with gold in the process. Steaming was carried out with a current of 20mA in duration of 90 seconds with a working distance of WD 50mm [19]. Recording and analysis were done on the Scanning electron microscope (JEOL JSM 6460LV) and connected by OXFORD INCA x-sight spectral analyzer. For the purposes of this analysis, the images were obtained by the Back-scatter or Primary emissions of reflected electrons in Compo mode (BEIc), as it proved to be the most useful for emphasizing enamel zones and dentine mass. The samples were observed at an acceleration of 20 kV at a working distance (WD) of 10 mm and at an inclination angle that was suitable for the inclination of the polished surface of the premolars and molars. The general image was given in a transparent magnification of 35x, The general image was given in a transparent magnification of 35x, and for the purposes of more precise EDS analysis, the

magnification of 100x was used. The obtained results were analyzed and statistically processed.

## 2.1. Statistical data processing

Qualitative data (mass fraction of elements) are shown by the number of phenomena and percentage representation. The differences in the mass fraction of the elements between the groups were tested by the Mann-Whitney U test.

## 3. RESULTS

Table 1 shows the average values of the mass fractions of: C (carbon), O (oxygen), Na (sodium), Mg (magnesium), Al (aluminum), Cl (chlorine), K (potassium), J (iodine) and Pb (plumbum) in the parts of the teeth in all examined groups.

The average values of the mass fraction of carbon in the teeth of rats receiving lead in the course of 14 days to whom diabetes was induced, was the highest in the area of enamel (24.25%), and the lowest in the area of enamel-dentine line (19.95%). In the rats receiving lead in the course of 30 days who were also brought into diabetes, the biggest mass fraction was found in the enamel (31.09%) and the smallest one in the pulp (17.83%).

The average value of oxygen content in rats' teeth that received lead in drinking water in the course of 14 who were brought into DM was the greatest in the area of dentine (39.43%) and the smallest in the enamel (38.17%). In the rats receiving lead in the course of 30 days who were brought into DM, the mass fraction was the largest in the area of the enamel-dentine line (44.88%), while the lowest one was in the pulp (36.52%).

The average value of the fraction of sodium in the teeth of rats receiving lead through drinking water in the course of 14 days, who were brought into DM was the highest in the area of dentine (0.22%), while it was not found in the area of enamel-dentine line. In the teeth of rats receiving lead in the course of 30 days, who were brought into DM, the mass fraction of sodium was the largest in the area of enamel (0.91%), while in the area of dentine and pulp was not detected at all.

The average value of magnesium in the teeth of rats that received lead in drinking water in the course of 14 days, who were brought into DM, was the highest in the area of dentine (0.11%) while it was not detected in other parts of the tooth. In the teeth of rats receiving lead in the course of 30 days, who were brought into DM, the mass fraction was the largest in the area of enamel (0.21%), while in

the enamel-dentine line, dentine and the pulp it was not detected at all.

The average value of the fraction of aluminum in the teeth of rats receiving lead through drinking water in the course of 14 days, who were brought into DM, was only detected in the area of enamel-dentine line (0.07%), while in the other parts of the tooth, it was not detected. In the teeth of rats receiving lead in the course of 30 days, who were brought into DM, the mass fraction of aluminum was not found in any examined part of the tooth.

The average value of the fraction of chlorine in the teeth of rats receiving lead through drinking water in the course of 14 days, who were brought into DM, was the highest in the area of dentine (0.21%) and the lowest in the area of enamel-dentine line (0.00%). In the teeth of rats receiving lead in the course of 30 days, who were brought into DM, the mass fraction of chlorine was only detected in the layer of enamel-dentine line (0,02) while in the other parts of tooth it was not found.

Potassium was not detected in the teeth of rats receiving lead through drinking water in the course of 14 days, who were brought into DM, in any of the

layers. In the teeth of rats receiving lead in the course of 30 days, who were brought into DM, potassium was only detected in the area of enamel (0.08%), while in the enamel-dentine line, dentine and the pulp, it was not detected at all.

In the teeth of rats receiving lead through drinking water in the course of 14 days, who were brought into DM, lead was not detected in any layer of the teeth. In the teeth of rats receiving lead in the course of 30 days, it was only detected in the enamel (0,36).

This analysis determined the mass fraction of the elements in the indicated parts of the teeth and in the indicated segments, therefore the presence of the following elements was found: C (carbon), O (oxygen), Na (sodium), Mg (magnesium), Al (aluminum), Cl (chlorine), K (potassium), J (iodine) and Pb (lead).

In the following Tables (Table 1 and 2), the number of positive findings of mass fractions of the mentioned elements was found in the examined parts of the teeth in both examined groups (A1 and A2 group).

Table 1. The average values of the mass fraction of certain elements in the teeth parts in the examined groups

	Spectrum	Group											
		Pb 14 days						Pb 30 days					
		N		SD	Med	Min	Max	N		SD	Med	Min	Max
Wt% C	Enamel	12	24.25	13.57	20.86	9.12	53.65	7	31.09	15.17	28.94	13.53	56.63
	EDJ	11	19.95	6.92	18.89	11.74	35.28	8	21.68	9.52	17.58	14.95	43.24
	Dentine	12	23.56	6.58	24.87	11.57	32.36	6	19.91	6.78	22.61	10.08	26.09
	Pulp	0	.	.	.	.	.	1	17.83	.	17.83	17.83	17.83
	Total	35	22.66	9.55	21.25	9.12	53.65	22	24.02	11.50	21.68	10.08	56.63
Wt% O	Enamel	12	38.17	8.26	41.80	16.78	43.88	7	41.13	9.63	44.84	24.75	54.55
	EDJ	11	38.26	4.48	38.81	31.09	44.79	8	44.88	8.74	46.87	27.69	54.48
	Dentine	12	39.43	5.90	39.62	30.00	46.61	6	44.14	11.07	46.39	24.26	55.13
	Pulp	0	.	.	.	.	.	1	36.52	.	36.52	36.52	36.52
	Total	35	38.63	6.29	39.58	16.78	46.61	22	43.10	9.28	45.05	24.26	55.13
Wt% Na	Enamel	12	.09	.32	.00	.00	1.10	7	.91	1.22	.82	.00	3.44
	EDJ	11	.00	.00	.00	.00	.00	8	.22	.41	.00	.00	.98
	Dentin	12	.22	.51	.00	.00	1.35	6	.00	.00	.00	.00	.00
	Pulp	0	.	.	.	.	.	1	.00	.	.00	.00	.00
	Total	35	.11	.35	.00	.00	1.35	22	.37	.79	.00	.00	3.44
Wt% Mg	Enamel	12	.00	.00	.00	.00	.00	7	.21	.00	.00	.00	.00
	EDJ	11	.00	.00	.00	.00	.00	8	.07	.21	.00	.00	.58
	Dentine	12	.11	.27	.00	.00	.81	6	.00	.00	.00	.00	.00
	Pulp	0	.	.	.	.	.	1	.00	.	.00	.00	.00
	Total	35	.04	.16	.00	.00	.81	22	.03	.12	.00	.00	.58
Wt% Al	Enamel	12	.00	.00	.00	.00	.00	7	.00	.00	.00	.00	.00

	Spectrum	Group											
		Pb 14 days						Pb 30 days					
		N		SD	Med	Min	Max	N		SD	Med	Min	Max
	EDJ	11	.07	.22	.00	.00	.74	8	.00	.00	.00	.00	.00
	Dentine	12	.00	.00	.00	.00	.00	6	.00	.00	.00	.00	.00
	Pulp	0	.	.	.	.	.	1	.00	.	.00	.00	.00
	Total	35	.02	.13	.00	.00	.74	22	.00	.00	.00	.00	.00

Table 2. The average values of the mass fraction of certain elements in the teeth parts in the examined groups

	Spectrum	Group											
		Pb 14 days						Pb 30 days					
		N		SD	Med	Min	Max	N		SD	Med	Min	Max
Wt% Cl	Enamel	12	.21	.41	.00	.00	1.16	7	.22	.30	.00	.00	.77
	EDJ	11	.00	.00	.00	.00	.00	8	.02	.07	.00	.00	.19
	Dentine	12	.16	.37	.00	.00	1.03	6	.00	.00	.00	.00	.00
	Pulp	0	.	.	.	.	.	1	.00	.	.00	.00	.00
	Total	35	.13	.33	.00	.00	1.16	22	.08	.19	.00	.00	.77
Wt% K	Enamel	12	.00	.00	.00	.00	.00	7	.03	.08	.00	.00	.20
	EDJ	11	.00	.00	.00	.00	.00	8	.08	.22	.00	.00	.62
	Dentine	12	.00	.00	.00	.00	.00	6	.00	.00	.00	.00	.00
	Pulp	0	.	.	.	.	.	1	.00	.	.00	.00	.00
	Total	35	.00	.00	.00	.00	.00	22	.04	.14	.00	.00	.62
Wt% I	Enamel	12	.32	.61	.00	.00	1.70	7	.17	.45	.00	.00	1.20
	EDJ	11	.46	.80	.00	.00	2.10	8	.00	.00	.00	.00	.00
	Dentine	12	.18	.62	.00	.00	2.14	6	.27	.65	.00	.00	1.60
	Pulp	0	.	.	.	.	.	1	.00	.	.00	.00	.00
	Total	35	.32	.67	.00	.00	2.14	22	.13	.42	.00	.00	1.60
Wt% Pb	Enamel	12	.00	.00	.00	.00	.00	7	.36	.62	.00	.00	1.30
	EDJ	11	.00	.00	.00	.00	.00	8	.00	.00	.00	.00	.00
	Dentine	12	.00	.00	.00	.00	.00	6	.00	.00	.00	.00	.00
	Pulp	0	.	.	.	.	.	1	.00	.	.00	.00	.00
	Total	35	.00	.00	.00	.00	.00	22	.11	.37	.00	.00	1.30

The differences in the mass fraction of the elements between the groups were tested by Mann-Whitney U test. Statistically significant difference was not determined between mass fractions of the examined elements (Table 1 and 2).

#### 4. DISCUSSION

Despite the fact that levels of lead in hard tooth tissues are useful indicators of lead exposure, information on its time effects and lead compounds in the tissue of the tooth is very limited [2]. The

basis of lead toxicity is that its metal cations are bound to special ligands (eg. sulphide, carboxylic or amino groups) of biomolecular substances that are of particular importance for different physiological functions and ion transport [20]. Although it does not belong to the pulpo-dentine complex, enamel as the most mineralized tissue in the body makes the protective cover of the pulpo-dentine complex in the area of the crown of a tooth. Deficit of essential elements increases the toxicity of heavy metals, while surplus acts protectively. A large part of this research was performed on laboratory animals, i.e. their bones and teeth where they accumulate most,

while data on humans are scarce, but mostly identical to those performed on animals [21]. Encouraged by this finding, this study was concerned with the study of the influence of lead and diabetes on the teeth of rats and their relation to the elements that enter the structure of the teeth. The results of this experimental study have indicated that the average values of the mass fraction of carbon, oxygen, sodium, magnesium, chlorine, potassium, iodine and lead were slightly higher in the area of tooth enamel of the rats receiving lead in the course of 30 days, with induced diabetes. There was no statistically significant difference between the mass fractions of the mentioned elements that were detected in the teeth samples of both experimental groups.

The results of this study are also consistent with the findings of the Baranowska-Bosiack study, who, with her associates (2008) examined the effect of melatonin on male Vistar strains, which, from birth to full maturity, took lead-acetate in drinking water and melatonin in food. The value of Pb, Ca and Mg in the teeth was measured by atomic absorption spectrophotometry.

In rats that took both lead and melatonin, a significant increase of lead in the blood, bones and teeth has been noticed. There Ca and Mg concentrations in the teeth of the rats were also determined, i.e. in enamel with no statistically significant difference [22]. Deposited lead in hard tissues, according to some authors, does not necessarily represent a danger to the organism, nor must have toxic effects on them, except in cases of rapid mobilization and transition to blood due to certain physiological or pathological processes, for example, pregnancy, growth and development of children [23]. The relationship between lead and the metabolism of bioelements was examined with special attention. Antila examined the absolute concentration of Mn, Fe, Ni, Cu, Zn, Sr and Pb in the enamel (from the labial and lingual side, lower deciduous incisors) by using proton induction with x-rays. Significant variations in concentration from the oral and labial surfaces of individual teeth were detected, only for lead, and for other metals there was no statistically significant difference for lingual and oral surfaces, which coincides with our study where no statistically significant difference, was noted [24]. Barmes found correlation of lead concentration in the teeth and tooth decay, more precisely the subjects with high lead concentration had more teeth with dental caries compared to those with lower level of lead [25]. Some studies suggest that there is a correlation between the presence of lead in the dental tissue and clinical changes in

enamel. Such changes can be associated with discoloration. However, this study was not sufficient to establish a clear correlation between the presence of lead and such damages of the enamel [26].

Results that were not in accordance with our findings are the results by the Grobler et al. who examined pregnant female rats, who received lead in drinking water during pregnancy and during lactation. The lead concentration in molars was investigated by atomic absorption spectrophotometry and it was found that the lead was mostly incorporated into the tooth tissue of females that drank water with the highest lead concentration [27]. Unlike our study, this study was performed by using high concentrations of lead in water that is not normally found in the nature. Lead poisoning is much faster in children and infants of animals, which is a consequence of a greater degree of resorption from the digestive tract.

It has already been noted that 50% of the orally taken amount of lead is resorbed in children, while in adult resorption is significantly smaller and amounts to only 10% [20]. The above can justify the result obtained in our study where a small mass fraction of lead (0.36) was detected in the area of enamel of the teeth of rats receiving lead in the course of thirty days through drinking water. In our experimental rats, the lead resorption was slower from the gastrointestinal tract compared to the previous study in young adult rats in which this resorption was increased by 50% and consequently there was no significant incorporation of lead in the teeth of rats. However, the results of one of the studies were not entirely in accordance with our results where the lead was detected in the enamel. Youravong et al. examined enamel and dentine in children with high levels of lead in the blood by using the method of secondary ion mass spectrometry –SIMS and x-rays microanalysis. These methods indicated the visible level of lead in the dentine-pulp line and microanalysis with x-rays could not detect lead. Microanalysis with x-rays in this study was unable to detect lead as opposed to secondary ionic mass spectrometry which detected it in dentine-pulp line [28]. In our study, it was also assumed that a higher concentration of lead could be detected by a more sensitive and precise method in other parts of the teeth, not only in the enamel. One of them is SEM with multi-element detector which can be a good substitute for conventional chemical testing, especially when it comes to Ambient SEM, which is easy to handle, with a short-term analysis that does not require standard protocols for sample preparation, which is usually required when it comes to SEM analysis [29].

Increased lead concentrations can also affect the development of children and the nutrient resorption, such as calcium, selenium, copper, iron and magnesium, as well as reduced activity of different cell enzymes. Lead is normally accumulated mainly in the bones and teeth. It is known that metals are incorporated in dental tissues during the period from the formation of enamel or dentine (during the teeth formation) [8,23]. There are reports in the literature that indicate that the presence of lead in the chemical composition of the enamel can alter its dental ultrastructure and lead to enamel damage. Thus, Gomes et al. in his study found that the teeth of pre-school children who lived in the industrial area of the city had higher concentration of lead in the enamel, compared to the teeth of children living in the non-industrial area [9,30].

Grobleret al. were testing pregnant female rats receiving lead through drinking water, during the pregnancy and lactation. Concentration of lead in molars was tested by using atomic absorption spectrophotometry and it was found that lead was mostly incorporated in dental tissue in females who drank water with the highest concentration of lead. However, this study was performed by using high concentrations of lead in water that is not normally found in the nature. It has also been confirmed that hypoplasia of enamel was increased in children exposed to high levels of lead, which may slightly justify our study having low concentration of lead and a result in which no statistically significant differences were observed [27]. It is interesting that with the incisors of the rats exposed to lead, a delayed dentition of incisors was observed. An increase in protein in the matrix of the enamel of these rats could be associated with the effect of stopping growth. Lead is a cytotoxic agent and changes in the amount of protein and delay in amelogenesis could be associated with the direct affect of lead on ameloblasts. The results indicate that mineralization is delayed due to exposure to lead [1,26].

However, Curzon and Vulovic did not notice the dependence of lead content in dental tissues and caries. There is no clear correlation between lead content in teeth and dental defect in Vulovic's research [31,32].

Diabetes mellitus is a chronic disease that negatively affects the repair of pulp (reparative ability of the pulp) and periodontium, as confirmed by numerous studies. Uncontrolled or inadequately controlled diabetes mellitus may be a risk factor for the development of oral complications. This prompted Catanzaro et al. to investigate whether

DM leads to inflammation and structural changes in the pulp. Male Wistar rats were injected streptozotocin injectionscoja (STZ) and induced artificial diabetes. After 30 and 90 days, nitrites in pulp tissue were elevated only in the first 30 days, and after 60 days they were lower in relation to the control group. This study suggests that diabetes leads to changes in oral tissues and to expression of the inflammatory mediator [33].

The following research is very interesting about influence of surface modification to mechanical and thermal properties of nanomodified acrylic dental resin [34] and residual monomer in dental acrylic resin and its adverse effects [35].

## 5. CONCLUSION

Mass fraction of biogenic elements found in the tooth enamel of the rats receiving lead in drinking water in the course of 30 days were slightly higher, but without a statistically significant difference with respect to the mass fraction of the elements in the group of rats receiving lead in drinking water in the course of 14 days. The lead was detected in the tooth of rats that received lead in the course of 30 days, but only in the enamel.

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#### АНАЛИЗА МАСЕНИХ УДЈЕЛА БИОГЕНИХ ЕЛЕМЕНАТА У ЗУБНОМ ТКИВУ

**Сажетак:** Увод: Чврста зубна ткива представљају идеална ткива за процјену дугорочних ефеката излагања организма токсичним металима. Циљ овог рада је био да се SEM-EDS анализом одреди заступљеност и међусобни однос масених удјела сљедећих елемената: угљеника, кисеоника, натријума, магнезијума, алуминијума, хлора, калијума, јода и олова у одређеним дијеловима зуба (глеђно-дентинска граница, дентин и пулпа) пацова са експериментално изазваним дијабетес мелитусом (ДМ), након 14 и 30 дана излагања животиња олову.

Материјал и методе рада: Истраживање је спроведено код пацова Wistar соја подијелених у двије групе. Прву експерименталну групу (А1) је чинило осам пацова, који су узимали олово током 14 дана у концентрацији од 1500 ppm, а другу експерименталну (А2) групу је чинило осам пацова који су узимали олово током 30 дана у концентрацији од 1500 ppm. Групе пацова А1 и А2 су уведене у дијабетес мелитус помоћу раствора Аллохан-а, који је апликован интраперитонеално у дози од 100 mg на килограм тјелесне тежине. Узорци зуба су анализирани скенинг електронском микроскопијом (SEM).

Резултати: У зубима пацова који су добијали олово у води за пиће током 14 дана и који су уведени у дијабетес мелитус, није детектовано олово, док су просјечне вриједности масених удјела осталих испитиваних елемената износиле: угљеник – 24,25%, кисеоник – 38,17%, натријум – 0,9%, магнезијум – 0,11%, алуминијум – 0,07%, хлор – 0,21% и јод – 0,32%. Просјечне вриједности масених удјела испитиваних елемената у глеђи зуба пацова који су добијали олово 30 дана и који су доведени у дијабетес мелитус, износиле су: олово – 0,36%, угљеник – 31,09%, кисеоник – 41,13%, натријум – 0,91%, магнезијум – 0,21%, хлор – 0,22%, калијум – 0,03% и јод – 0,17%.

Закључак: Масени удјели елемената који су пронађени у глеђи зуба пацова који су добијали олово у води за пиће током 30 дана и доведени у дијабетес, били су нешто већи али без статистички значајне разлике у односу на масене удјеле елемената код групе зуба пацова који су добијали олово у води за пиће 14 дана и који су били доведени у дијабетес, такође. Олово је детектовано у зубима пацова који су добијали олово током 30 дана и доведени у дијабетес и то само у глеђи.

**Кључне ријечи:** масени удјели елемената глеђи; дентиногенеза; SEM-EDS анализа.

