

# 15.\_The\_Impact\_of\_Cadmium\_Exposure\_on\_Several\_Tooth.pdf

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## The Impact of Cadmium Exposure on Several Tooth Mineral Content

Diny Aulia Ratnasari<sup>1\*</sup>, M. Rizal Surya Permana<sup>1</sup>, Widodo<sup>1</sup>, Yanuar Ichrom N<sup>1</sup>, Nurdiana Dewi<sup>1</sup>, Eko Suhartono<sup>2</sup>

<sup>1</sup>Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia

<sup>2</sup>Department of Medical Chemistry/Biochemistry, Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia

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### ABSTRACT

The objectives of this study were to determine the impact of cadmium (Cd) on several tooth mineral content. The tooth mineral content that investigated in this present study are calcium (Ca), Phosphate (P), magnesium (Mg), and zinc (Zn). Teeth samples were taken from 25 human maxillary premolar-1 free of caries and defects. All the teeth samples were extracted in Dental Faculty, University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia. Each of tooth sample was put in the water contained Cd in the form of cadmium sulphat (CdSO<sub>4</sub>) with concentration 1 mg/l for 96 hours. The mineral content of the tooth estimation were made from 0 hours and every 24 hour, so the teeth samples can be divided into 5 groups based on the time of Cd exposure (T1: 0 hours; T2: 24 hours; T3: 48 hours; T4: 72 hours; and T5: 96 hours). The results of this present study show that the administration of Cd led to a significant decrease of Ca, P, Mg, and Zn level in the tooth. The results suggest that Cd exposure can affect several mineral content in the tooth, including Ca, P, Mg, and Zn.

**Keywords:** Cadmium, Calcium, Magnesium, Phosphate, Tooth, Zinc

### INTRODUCTION

Cadmium (Cd) is well-known toxic metals that are widespread in the environment<sup>1</sup>. This metal is between zinc (Zn) and mercury (Hg) in the periodic table. The chemical behavior of Cd is similar to Zn. It generally exists as the divalent cation, complexes with other elements (e.g., CdCl<sub>2</sub>). Industrially, Cd is used in electro-plates manufacture, batteries, alloys, and fuels<sup>2</sup>. Approximately 13,000 tons of Cd is produced worldwide each year<sup>3</sup>.

Cd has high rates of soil-to-plant transfer, making diet as a primary source of exposure<sup>4,5</sup>. After absorption, Cd is transported throughout the body, and irreversibly accumulates in several organs such as liver and kidney, with clearance half-life of Cd is about twenty-five years<sup>6-7</sup>. It may further lead several human health problems, such as hepatic and renal tubular diseases, obstructive pulmonary disease, skeletal disorders, and cardiovascular diseases<sup>8-10</sup>.

It is well documented in our previous study, Cd could affect several organs, both in vitro and in vivo condition. The oral administration of Cd (3 mg/l) in rats for 4 weeks induced ovarian damage, which was evident significantly increased levels of ovarian methylglyoxal, hydrogen peroxide, Advanced Oxidation Protein products (AOPPs), and a carbonyl compound<sup>11</sup>. Also, the oral administration of Cd with the same doses and time induced kidney damaged, which was evident significantly increased levels of kidney malondialdehyde, hydrogen peroxide, AOPPs, methylglyoxal, and carbonyl compound<sup>3</sup>.

Some investigators suggest that environmental exposure of Cd was associated with tooth decay. Amr et al<sup>12</sup> found that Cd level in the tooth is associated with caries. The Cd also known can cause several histopathology changes of the tooth<sup>13</sup>. However, till now there is a lack of study on the effects of Cd to the release of several dental minerals. Thus, our present study aimed to investigate the effect of Cd to the release of several dental minerals, such as calcium (Ca), phosphate (P), magnesium (Mg), and Zn.

### MATERIAL AND METHODS

The present study was a true experimental study with post test only with control group design to examine the impact of Cd on several mineral content (Ca, P, Mg, and Zn) in the tooth. Tooth put in water containing Cd in the form of cadmium sulphate (CdSO<sub>4</sub>) with concentration 1 mg/L for 0-96 hours. Observation was made at 0, 24, 48, 72, and 96 hours and each time the levels of Ca, P, Mg, and Zn was examined.

#### Samples Preparation

Before the preparation of samples, ethical approval was obtained from the Ethics Committee of the Faculty of Medicine of Lambung Mangkurat University. Tooth samples were prepared from 25 extracted human maxillary premolar 1 free of caries and defects. All tooth samples were collected from the Dental Faculty of Lambung Mangkurat University, Banjarmasin, Indonesia. After the extraction, the teeth were dried in the oven at 100°C temperature for 2 days.

#### Experimental Section

\*Author for Correspondence: [ekoantioxidant@gmail.com](mailto:ekoantioxidant@gmail.com)

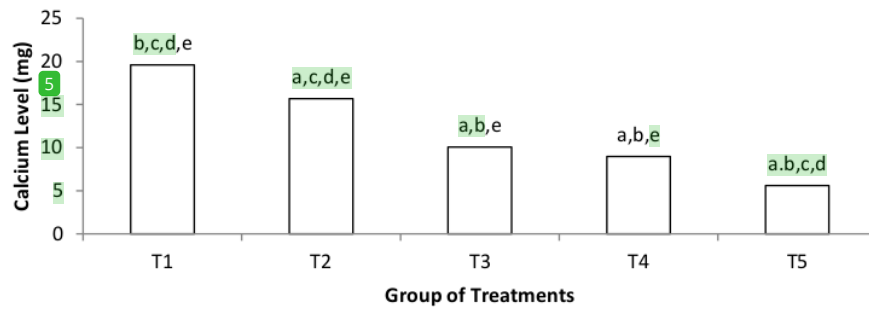


Figure 1: Teeth calcium level in a different group of treatments. Values are mean±SEM of three replicates in each group of treatment. a: Significantly different when compared to Group T1; b: Significantly different when compared to Group T2; c: Significantly different when compared to Group T3; d: Significantly different when compared to Group T4; e: Significantly different when compared to Group T5. Comparison of variables between the groups was performed with Kruskal-Wallis test and Mann-Whitney U test (P<0.05).

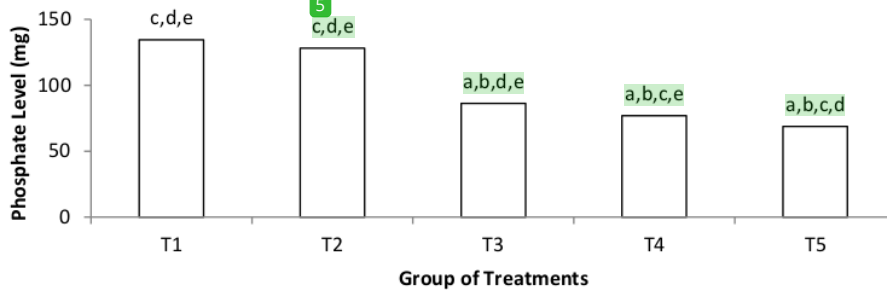


Figure 2: Teeth phosphate level in a different group of treatments. Values are mean±SEM of three replicates in each group of treatment. a: Significantly different when compared to Group T1; b: Significantly different when compared to Group T2; c: Significantly different when compared to Group T3; d: Significantly different when compared to Group T4; e: Significantly different when compared to Group T5. Comparison of variables between the groups was performed with Kruskal-Wallis test and Mann-Whitney U test (P<0.05).

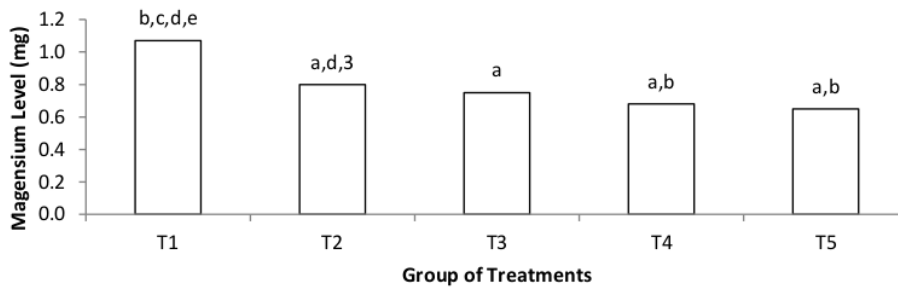


Figure 3: Tooth magnesium level in a different group of treatments. Values are mean±SEM of three replicates in each group of treatment. a: Significantly different when compared to Group T1; b: Significantly different when compared to Group T2; c: Significantly different when compared to Group T3; d: Significantly different when compared to Group T4; e: Significantly different when compared to Group T5. Comparison of variables between the groups was performed with Kruskal-Wallis test and Mann-Whitney U test (P<0.05).

Teeth samples were divided into 5 groups with 5 samples of teeth in each group. Treatment 1 (T1) group: The teeth samples were submerged in Cd-contained water for 0 hours; Treatment 2 (T2) group: The teeth samples were submerged in Cd-contained water for 24 hours; Treatment

3 (T3) group: The teeth samples were submerged in Cd-contained water for 48 hours; Treatment 4 (T4) group: The teeth samples were submerged in Cd-contained water for 72 hours; Treatment 5 (T5) group: The teeth samples were submerged in Cd-contained water for 96 hours. After

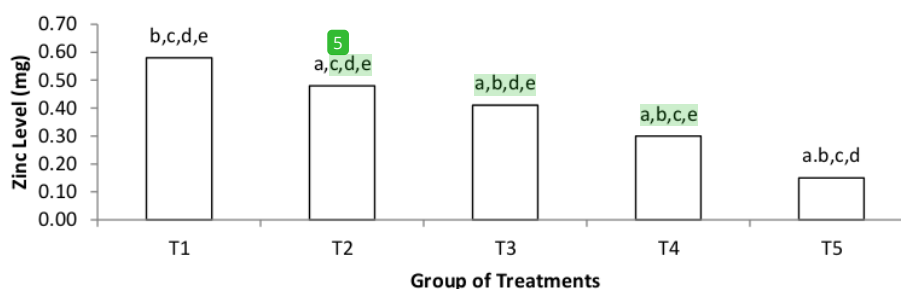


Figure 4.:Teeth zinc level in a different group of treatments. Values are mean $\pm$ SEM of three replicates in each group of treatment. a: Significantly different when compared to Group T1; b: Significantly different when compared to Group T2; c: Significantly different when compared to Group T3; d: Significantly different when compared to Group T4; e: Significantly different when compared to Group T5. Comparison of variables between the groups was performed with Kruskal-Wallis test and Mann-Whitney U test ( $P < 0.05$ ).

treatment, each sample was cleaned and crushed with mortar till homogenous and undergo to Ca, P, Mg, and Zn content analysis.

#### Calcium Content Analysis

The sample solution was added  $\text{NH}_4\text{OH}$ ,  $\text{HCl}$  (1:4), 0.5 M  $\text{HCl}$ , and 10 ml of 10% oxalic acid. The solution was heated to boiling and while stirring add 15 ml of  $\text{NH}_4$ -oxalate. Then filtered and washed with hot water until free of chloride. After that, add 10 mL of  $\text{H}_2\text{SO}_4$  and heated to boiling and then cooled. Titrated with 0.1 N  $\text{KMnO}_4$ <sup>14</sup>.

#### Phosphate Content Analysis

A total of 50 ml of the sample solution was added 2 ml of ammonium molybdate and 5 drops of  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ . Subsequently, the solution was put in a cuvette and measuring the spectrophotometric with  $\lambda = 590 \text{ nm}$ <sup>14</sup>.

#### Magnesium Content Analysis

Mg content analysis was performed using with complexometric titration with slight modifications.<sup>15</sup> Prepare a sample solution by diluting 250 mg sample with 100 ml aquadest. Add 3 N hydrogen chloride ( $\text{HCL}$ ) to the sample solution until the pH of the solution is almost 7 (according to pH indicator paper). Then, add 1 N sodium hydroxide ( $\text{NaOH}$ ), 5 ml buffer ammonia, and 0.15 ml eriochrome black T solution. Titrate the sample solution with 0,05 M disodium edetate till the color of the solution changed to blue.

#### Zinc Content Analysis

A total of 25 ml of the sample solution was added 15 ml of aqua deist, 10 ml of ammonia buffer pH 10 and EBT. Titration with  $\text{EDTA}$  0.01 M<sup>14</sup>.

#### Statistical Analysis

The results are expressed as the mean  $\pm$  SD for five replicates. The difference of Ca, P, Zn, and Mg content between groups of treatment was evaluated by Kruskal-Wallis test and followed by Mann-Whitney test. All data were entered into and processed by SPSS 16.0 for Windows.

## RESULTS

This present study, which was undertaken to assess the effects Cd to Ca, P, Zn, and Mg level in the human teeth.

The first mineral content that investigated in this present study was Ca. The result can be seen in figure 1. After treatment in Cd-contained water, there was a significant decrease in Ca levels in a group of treatments compared to control (Kruskal-Wallis test;  $P < 0.05$ ). Mann-Whitney test results show that there are significant differences between each group of treatments except between group T1-T4, and T3-T4.

The second mineral content that investigated in this present study was P. The P content in the teeth in the different group of treatments can be seen in figure 2. The result from figure 2 shows that P level in all treatment groups was lower than the control group. Kruskal-Wallis test analysis shows that there was a significant decrease in Fe level between a group of treatments ( $P < 0.05$ ). Mann-Whitney test results show that there is a significant difference between each group of treatments except between group T1 and T2.

The third mineral content that investigated in this present study was Mg. The Mg content in the teeth in the different group of treatments can be seen in figure 3. The result from figure 3 shows that Mg level in all treatment groups was lower than the control group. Kruskal-Wallis test analysis shows that there was a significant decrease in the Mg level in a group of treatments compared to the control group ( $P < 0.05$ ). Mann-Whitney test results show that there is a significant difference between a group of treatments except between T2-T3, T3-T4, T3-T5, and T4-T5.

The fourth mineral content that investigated in this present study was Zn. The Zn content in the teeth in the different group of treatments can be seen in figure 4. The result from figure 4 shows that Zn level in all treatment groups was lower than the control group. Kruskal-Wallis test analysis shows that there was a significant decrease in Zn level in a group of treatments compared to the control group ( $P < 0.05$ ). Mann-Whitney test results show that there is a significant difference between each group of treatments.

## DISCUSSION

Cd is one of the heavy metals that has great effects on human health (Suhartono IJPPS 2015). Exposure to Cd can

damage various organs including lung, liver, kidney, bone, testis, nervous system, placenta, and tooth<sup>12,16-18</sup>. Results of this present study indicated that Cd exposure affects several mineral content in the tooth.

Tooth is one of the most highly mineralized tissue in the human body. The primarily mineral content in the tooth are Ca and P in the form of hydroxyapatite. Also, the tooth contains other trace mineral such as Mn, Fe, Mg, F, Cl, K, Sn, Sr, Ni, Co, Cr. The mineral content of the tooth is varied from person to person, and indeed from tooth to tooth<sup>14,19</sup>.

The mineral content of the tooth can affect by several factors, including saliva condition, acid beverages, acidic foods and dietary ingredients, and heavy metal exposure<sup>20-21</sup>. Results of our present study indicated that Cd exposure can affect several mineral content in the tooth. The first mineral that investigated in this present study is Ca. As mentioned above, Ca is one of the primary mineral content in the tooth. Also, it is well known that Ca has the same valence numbers with Cd, i.e. 2+. Also, the Cd has a similar physical and chemical characteristics with Ca. Those characteristics can make Cd displace Ca in tooth hydroxyapatite and result in the decreasing of Ca content in the tooth<sup>22</sup>.

The second mineral content that investigated in this present study is P. P another primarily mineral content in the tooth hydroxyapatite. This mineral is tightly bond with Ca in tooth hydroxyapatite. As mentioned above, Cd can replace Ca in tooth hydroxyapatite. Therefore, the Cd can also bind to P in tooth hydroxyapatite. This bind will be led to the dissolution of crystal hydroxyapatite, so the level of P is decreased in the tooth<sup>23</sup>.

The third mineral content that investigated in this present study is Mg. The basic mechanism how Cd can affect the Mg content in the tooth is the same reason why Cd can affect the Ca content in the tooth. Cd has the same valence number with Mg. From this point of view, Cd also has an ability to replace Mg in the tooth. Furthermore, Mg also known can prevent tooth decay by taking Ca in tooth enamel. When the position of Mg in the tooth is replaced by Cd, this will lead to the demineralization of the tooth<sup>24</sup>.

The fourth mineral content that investigated in this present study is Zn. Zn is an essential trace element, and the human body has efficient mechanisms, both on systemic and cellular levels, to maintain homeostasis over a broad exposure range<sup>25</sup>. The human body contains 2–3 g Zn, and nearly 90% is found in muscle and bone. Other organs containing estimable concentrations of Zn include prostate, liver, the gastrointestinal tract, kidney, skin, lung, brain, heart, pancreas, and tooth<sup>12,25</sup>.

Based on our results study, Cd exposure can decrease the Zn content in the tooth. According to the biochemistry of heavy metal toxicity, a metal ion in the body can be conveniently replaced by another metal ion of similar size<sup>26</sup>. In the periodic table of the elements, Zn can be found in group IIb, together with the two toxic metals Cd and mercury. This makes Zn have a similar physical and chemical properties. From this point of view, Cd can replace Zn because of this similarity<sup>25-26</sup>. Thus, Cd could decrease the Zn content in the tooth.

In conclusion, the present study demonstrated that the Cd exposure can affect the tooth mineral content, including Ca, P, Mg, and Zn. It is indicated that the exposure of peat swamp water can cause the demineralization of the tooth minerals, such as Ca, P, Mg, and Zn.

#### CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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