

# 18.\_Demineralization\_of\_the\_To oth.pdf

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## Demineralization of the Tooth by Peat Swamp Water

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### ABSTRACT

In this present study, we try to demonstrate the effect of peat swamp water to several mineral content, such as manganese (Mn), iron (Fe), magnesium (Mg), and fluor (F) in a tooth. Tooth samples were taken from 25 human maxillary premolar 1 free of caries and defects. All the tooth samples were extracted in Dental Faculty, University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia. Water sample is taken from peat swamp area in Anjir Pasar village with pH value around 3.5. Tooth samples then divided into five groups with; C served as control; T1 which submerge the tooth for 24 hours; T2 which submerge the tooth for 48 hours; T3 which submerge the tooth for 72 hours; and T4 which submerge the tooth for 96 hours. After treatment, each sample were cleaned and crushed with mortar till homogenous and undergo to Mg, Fe, Mn, and F content analysis. Overall, the tooth submersion in peat swamp water decreased the Mn, Fe, Mg, and F content in the tooth. The results of this present study indicated that exposure to peat swamp water could caused the demineralization of the tooth.

**Keywords:** Demineralization, Peat Swamp Water, Tooth

### INTRODUCTION

Peat is ecosystems characterized by the accumulation of partially decayed organic matter. This ecosystem occur at low altitudes in the river valley basins, watersheds, and subcoastal areas of Southeast Asia<sup>1</sup>. Indonesia is one of the countries which are located in Southeast Asia with the largest area of peat swamp forest, covering an estimated 20.7 mHa. Almost all Indonesia's peat swamp forest are located in three islands, Kalimantan, Irian Jaya, and Sumatera<sup>2</sup>. In Kalimantan, 8000 km<sup>2</sup> of peatland are located in South Kalimantan<sup>3</sup>. It is well known that the area of peat swamp is known by low water pH values and has extreme acidic environment<sup>4</sup>. This condition will lead to several health problems, including dental erosion<sup>5</sup>. Dental erosion is the loss of dental hard tissue, associated with extrinsic and/or intrinsic acid that is not produced by bacteria<sup>6</sup>. Several studies revealed the positive correlation between low pH in drinking water with both dental erosion and dental caries. These findings support the studies investigating the effect of acid drinking water on dental decay<sup>7</sup>. However, the pathomechanisms, diagnostic criteria, and preventive strategies of the condition are still not well established. But, it is believed that the dental erosion is caused by tooth enamel demineralization. In the oral environment, tooth structure undergoes continuous demineralization and remineralization: if this balance is interrupted, demineralization will lead to a progressive deterioration of tooth structure<sup>8</sup>. It is well known that the main structural component of the enamel is a mineral called hydroxyapatite which is chemically Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>

(OH)<sub>2</sub> (Castro, 2012). However, X-ray energy dispersive spectroscopy (EDS) analysis of enamel also indicated the presence in small quantities of other elements such as sodium (Na), chloride (Cl), manganese (Mn), iron (Fe), fluor (F) and magnesium (Mg)<sup>9,10</sup>. Previous study suggests that that chronic exposure to water in acid pH can cause demineralization of the tooth and resulted in a dental erosion. Considering the peat swamp water has a low pH and the people of South Kalimantan using this water for daily activities, knowledge of the effect of this water to dental mineral is substantial. Therefore, in this present study, we try to investigate the effect of peat swamp water exposure on dental mineral content, such as Mg, Fe, Mn, and F.

### MATERIAL AND METHODS

Samples collection and preparation: Before the samples collection, ethical approval was obtained from the Ethics Committee of the Faculty of Medicine of University of Lambung Mangkurat. 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. Water sample was taken and collected from peat swamp area in Anjir Pasar village, Banjar District, South Kalimantan, Indonesia. The water pH value is about 3.5. Experimental models: Samples were divided into 5 groups (1 control group and 4 treatment groups) on 5 sample tooth solution in each

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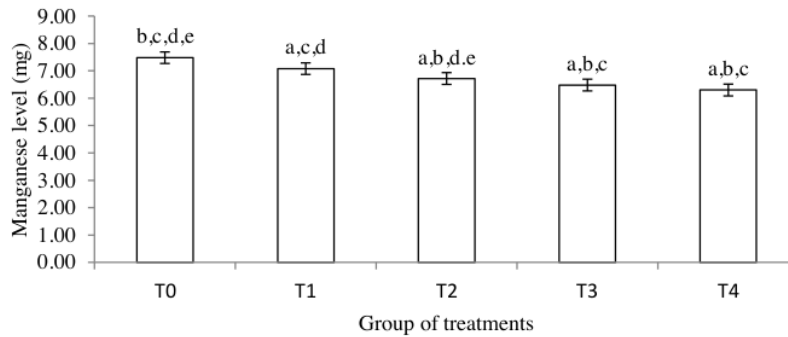


Figure 1: Tooth manganese level in different group of treatments. Values are mean±SEM of three replicates in each group of treatment.

a: Significantly different when compared to Group T0; b: Significantly different when compared to Group T1. c: Significantly different when compared to Group T2; d: Significantly different when compared to Group T3. e: Significantly different when compared to Group T4. Comparison of variables between the groups was performed with Kruskal-Wallis test and Mann-Whitney U test (P<0,05).

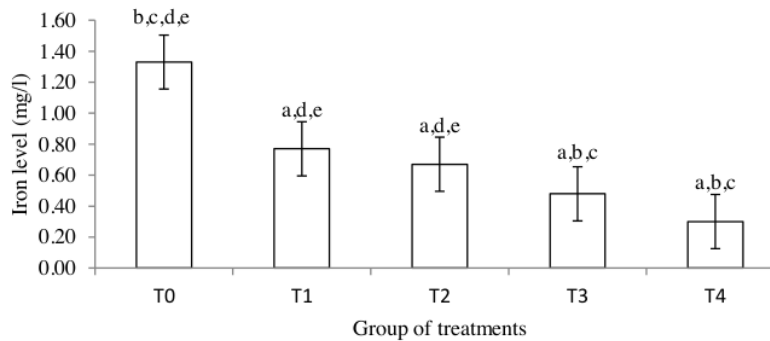


Figure 2: Tooth iron level in different group of treatments. Values are mean±SEM of three replicates in each group of treatment.

a: Significantly different when compared to Group T0; b: Significantly different when compared to Group T1. c: Significantly different when compared to Group T2; d: Significantly different when compared to Group T3. e: Significantly different when compared to Group T4. Comparison of variables between the groups was performed with Kruskal-Wallis test and Mann-Whitney U test (P<0,05).

group. Control (C) group: tooth without submerge in peat water; Treatment 1 (T1) group: tooth submerge in peat water for 24 hour; Treatment 2 (T2) group: tooth submerge in peat water for 48 hour; Treatment 3 (T3) group: tooth submerge in peat water for 72 hour; Treatment 4 (T4) group: tooth submerge in peat water for 96 hour. After treatment, each sample were cleaned and crushed with mortar till homogenous and undergo to Mg, Fe, Mn, and F content analysis. Magnesium content analysis: Mg content analysis was performed using with complexometric titration with slight modifications<sup>11</sup>. Prepare a sample solution by diluted 250 mg sample with 100 ml aquadest. Add 3 N hydrogen chloride (HCL) to sample solution until the pH of the solution is almost 7 (according to pH indicator paper). Then, add 1 N sodium hydroxide (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 colour of the solution changed to blue. Iron content analysis: Fe content analysis<sup>14</sup> was calculated using a spectrophotometer. Mix together 50 ml of the sample solution with 1 ml of hydroxylamine, and 2 ml of orto-phenantroline. Then, let the solution for 10 minutes and measured the absorbance of the solution using a spectrophotometer at a wavelength of 510 nm. Manganese content analysis: Mn content analysis was performed using a spectrophotometer. Prepare 0.1 mg sample into a flask. Then, add 5 mL of H<sub>3</sub>PO<sub>4</sub> and 0.5 g KIO<sub>3</sub> into a flask that contained tooth sample. Boiled for one minute and let it heat for 10 minutes. Measured the absorbance of the solution using a spectrophotometer at a wavelength of 560 nm. Fluor content analysis: The F content analyses were performed using an ion-meter in

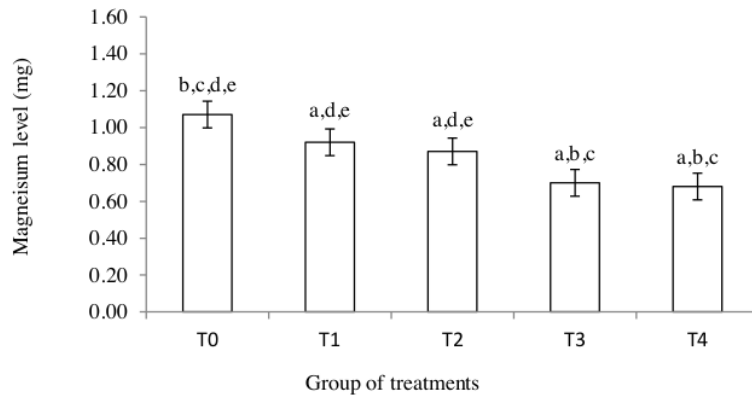


Figure 3: Tooth magnesium level in different group of treatments. Values are mean±SEM of three replicates in each group of treatment.

a: Significantly different when compared to Group T0; b: Significantly different when compared to Group T1. c: Significantly different when compared to Group T2; d: Significantly different when compared to Group T3. e: Significantly different when compared to Group T4. Comparison of variables between the groups was performed with Kruskal-Wallis test and Mann-Whitney U test (P<0,05).

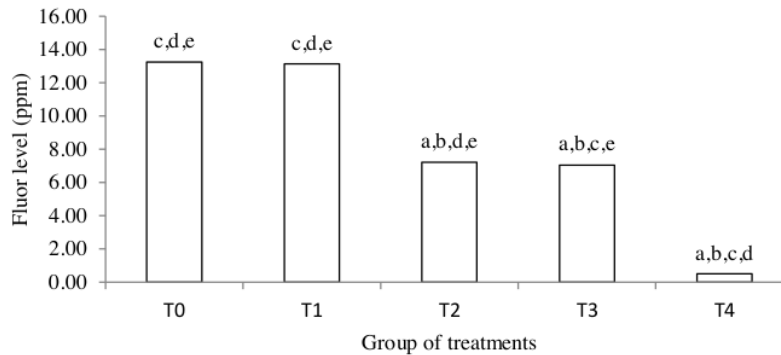


Figure 4: Tooth fluor level in different group of treatments. Values are mean±SEM of three replicates in each group of treatment.

a: Significantly different when compared to Group T0; b: Significantly different when compared to Group T1. c: Significantly different when compared to Group T2; d: Significantly different when compared to Group T3. e: Significantly different when compared to Group T4. Comparison of variables between the groups was performed with Kruskal-Wallis test and Mann-Whitney U test (P<0,05).

conjunction with a combination fluoride electrode<sup>12</sup>. The instrument calibration was performed using F standard solutions ranging in concentration from 0.05 to 2.0 ppm F<sup>-</sup> (prepared from 100.0 ppm F<sup>-</sup> fluoride standard) and TISAB (Total Ionic Strength Adjustor Buffer), a 1M acetate buffer, pH 5.0, 1.0 M NaCl, and 0.4% CDTA (1,2-Cyclohexylenedinitrilotetraacetic acid). The F standard solutions and the TISAB solution were used in the ratio 1:1 (v/v). The standards were chosen based on the desired F concentration in the samples<sup>13</sup>. The samples were analyzed after calibration of the ion-selective electrode coupled with the ion analyzer. The readings of the standards and the samples were performed under stirring using magnetic stirrer (MARTE) and magnet stir bars for one minute for

all samples. F concentration in the samples was determined according to the equipment operating procedure, and the accuracy of the results was evaluated using solutions of known concentration (test solutions), added every 3 readings. There was no difference greater than 3%. The data obtained in mV were inserted into a properly calibrated Excel spreadsheet for analysis and comparison of the results. The fluoride concentration results were obtained from linear regression of the calibration curve<sup>13</sup>. Statistical analysis: The results are expressed as the mean ± SD for three triplicates. The difference of Mn, Fe, Mg, and F 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 peat swamp water to Mn, Fe, Mg, and F level in the human tooth. The first mineral content that investigated in this present study was Mg. The result can be seen in figure 1. After the tooth soak in peat swamp water, there was a significance decrease in Mn level in group of treatments compared to control (Kruskal-Wallis test;  $P < 0.05$ ). Mann-Whitney test results shows that there are significant differences between each group of treatments except between group T1 and T4, and T3 and T4. The second mineral content that investigated in this present study was Fe. The Fe content in tooth in the different group of treatments can be seen in figure 2. Result from figure 2 shows that Fe level in all treatments groups were lower than the control group. Kruskal-Wallis test analysis show that there was a significance decrease in Fe level between group of treatments ( $P < 0.05$ ). Mann-whitney test results shows that there are a significant differences 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 tooth in the different group of treatments can be seen in figure 3. Result from figure 3 show that Mg level in all treatments groups were lower than the control group. Kruskal-Wallis test analysis show that there was a significance decrease in Mg level in group of treatments compared to the control group ( $P < 0.05$ ). Mann-whitney test results shows that there are a significant differences between group T0 and T1, T0 and T2, T0 and T3, T0 and T4, T1 and T3, T1 and T4, T2 and T3, and T2 and T4. The fourth mineral content that investigated in this present study was F. The result can be seen in figure 4. After the tooth soak in peat swamp water, there was a significance decrease in F level in group of treatments compared to the control group (Kruskal-Wallis test;  $P < 0.05$ ). Mann-Whitney test results shows that there are significant differences between group T0 and T2, T0 and T3, T0 and T4, T1 and T2, T1 and T3, T1 and T4, T2 and T3, T2 and T4, and T3 and T4. However, no difference were detected between group T0 and T1.

## DISCUSSION

One of the most highly mineralized tissue in the human body is the tooth enamel<sup>14</sup>. This tissue consists of approximately 96% minerals, 3% water, and 1% organic material by weight<sup>15</sup>. Mineral enamel contains a large amount of carbonate ions as well as a small percentage of trace elements such as: Mn, Fe, Mg, F, Cl, K, Sn, Sr, Ni, Co, Cr<sup>16,17</sup>. The highly mineral content in tooth enamel make this tissue strong and the other side makes it easy to break<sup>18</sup>. One of the conditions that can cause the breaking of tooth enamel is acid condition, especially for long exposure<sup>19</sup>. The breaking of tooth enamel by acidic condition induced a process, known as demineralization. Demineralization occurs at a low pH when the oral environment is under saturated with mineral ions, relative to a tooth's mineral content<sup>20</sup>. Acid which can cause tooth

demineralization may be extrinsic or intrinsic in origins, such as occupational exposure and lifestyle activities<sup>8</sup>. Peat swamp water is an acidic water. Exposure to the peat swamp water continuously can cause some health effects such as tooth demineralization<sup>17</sup>. This in line with the results of this present study. Dental submersion in peat swamp water can cause the decreasing of several mineral content in the tooth, including trace minerals, such as Mn, Fe, Mg, and F. Also, the results indicated that time of tooth submersion in peat swamp water affect the mineral content. It seems the mineral content are decreased with the increasing of tooth submersion time. The peat swamp water is known by low water pH values and has an extremely acidic environment (pH 4 or less)<sup>1,4</sup>. This condition will cause the mineral content in the tooth are dissolved. This is caused by the acidic conditions that cause the condition in the oral cavity is unsaturated. Then, the tooth mineral will dissolve to balance the saturation<sup>20</sup>. Based on our results the tooth submersion in peat swamp water led to decrease Mn and Fe content. Mn and Fe is a trace element that essential for many physiological processes including bone and tooth mineralization, protein and energy metabolism and free radical species production. Mn and Fe involvement in calcified tissues (bone and teeth) may be related to exposure to environmental factors<sup>21</sup>. It is well known that Mn and Fe is an electropositive chemical element and easily soluble in acid condition. Mn and Fe is unstable ion and easily oxidizes and reduces simultaneously. If the pH is below the critical pH, Mn and Fe will continue to dissolve until the solution becomes saturated<sup>22,23</sup>. Mg is one of a mineral component contained in the tooth enamel. Some 50 to 60% of the total body Mg content of approximately 25 g in the normal adult resides in bones and teeth. In this present study, tooth submersion in peat swamp water can lead to the decreasing of Mg content in the tooth. This might be caused by continuous exposure to acid. Exposure to acid will break the chemical bonds in the hydroxyapatite crystal. Acid contained H<sup>+</sup> ions. This ion can diffuse into the enamel and dentin that will damage the chemical bonds, including Mg<sup>24,25</sup>. Fluor is a chemical element that is highly electronegative. In the teeth, F found in very limited quantities. F in the tooth can be found as fluoro hydroxyapatites and fluorapatite which occupied in lattice intercrystalline of tooth enamel prism. It is well known that F is vulnerable to acid. This is in line with the the results of this present study. The result of this present study suggests that exposure to an acid derived from peat swamp water will lead to the decreasing of F content in tooth. This is presumably due to pressures of H<sup>+</sup> ions which contained in the peat swamp water. This pressure causes the F in fluorapatite are dissolved<sup>25</sup>. In conclusion, the present study demonstrated that the tooth submersion in peat swamp water can caused the tooth demineralization. Also, the present study demonstrated that there is a time depend decrease in tooth mineral content induced by submersion in peat swamp water. It is indicated that the exposure of peat swamp water can cause the demineralization of the tooth minerals, such as Mn, Fe, Mg, and F.

**CONFLICT OF INTEREST**

We declare that we have no conflict of interest.

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