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THE EFFECT OF PEAT SWAMP WATER ON TOOTH DEMINERALIZATION OF COPPER AND SELENIUM ION

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ABSTRACT
Background: *The society of South Kalimantan has high rate of tooth decay. The high rate of tooth decay in South Kalimantan is caused by environmental factors, namely water sources originating from peat swamp. Swamp water has acidic pH can make tooth demineralization or releasing minerals in tooth. One of the minerals that can be demineralized by swamp water is copper ions (Cu^{2+}) and selenium ions (Se^{4+}). The released minerals in tooth makes tooth enamel slowly dissolve and make tooth more susceptible to caries.* ***Objective:*** *to analyze the effect of tooth immersion on peat swamp water on releasing copper and selenium ions.* ***Methods:*** *This research used a laboratory experimental study with eighteen specimens of extracted human maxillary premolar teeth. The specimens were divided into 2 treatment groups ie tap (PDAM) water and peat swamp water immersion, and 1 control group ie artificial saliva immersion. Tooth immersion is conducted for 7 days. The Measurement of copper and selenium ions release using a UV-Vis spectrophotometer. Data were statistically tested using One Way Anova.* ***Result:*** *The mean value of released copper ions in artificial saliva (3.8 ± 0.51 ppm), tap water (3.72 ± 1.02 ppm) and peat swamp water (3.37 ± 0.97 ppm) showed there were no significant differences among all immersion groups. Meanwhile, the mean value of released selenium ions in artificial saliva (7.61 ± 2.34 ppm), tap water (2.4 ± 0.62 ppm) and peat swamp water (0.85 ± 0,38 ppm) showed there were significant differences among all immersion groups on the release of selenium ions, subsequenty test using Post hoc Dunnett's T3 showed p=0.0001 (p<0.05).* ***Conclusion:*** *There is no effect of tooth immersion in peat swamp water on the release of copper ions however there is an effect on the release of selenium ions.*

Keywords: *Demineralization, Peat swamp water, Releasing copper ions (Cu^{2+}), Releasing selenium ions (Se^{4+}), tooth decay.*

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INTRODUCTION

One of the most common dental and oral diseases in Indonesian are caries.[1] The DMF-T index in Indonesia is 4.6. The province of South Kalimantan has a DMF-T index of 7.2, which is greater than the DMF-T index in Indonesia. This indicates that the level of tooth decay in South Kalimantan is very high.[2] Based on RISKESDAS 2018, the people of South Kalimantan have dental and mouth problems above the average, which is almost 60%. The high rate of tooth decay in South Kalimantan is caused by environmental factors, particularly water sources originating from peat swamp land.[2,3]

The distribution of peat swamps in South Kalimantan is quite extensive, 106,271 ha. This condition causes many people in South Kalimantan to still use water sourced from peat swamps for daily needs such as gargling and brushing their teeth.[2] Peat swamp water or peat water is water that is widespread in swamp and lowland areas especially in Sumatra and Kalimantan, which has a low pH characteristic (3-5) or a high acidity level. Peat water has high organic content and high color intensity. Peat water also has a low content of macro elements such as Ca, P, Mg and K and low micro elements such as Cu, Se Mn, Zn and B. The high level of acidity of peat water is caused by the large amount of organic content ie humic acid, fulvic acid and humin.[4,5] Acidic condition in peat

water have a role in the process of tooth decay, the use of acidic water for gargling or toothbrushes can cause a decrease in surface hardness of tooth enamel or tooth demineralization.[5,7]

Tooth demineralization is the process of releasing minerals contained in the tooth.[7] Tooth have four constituent tissues, there are enamel, dentine, cementum and pulp. Enamel and dentine are composed of inorganic materials in the form of minerals, organic material and water. The most contained minerals contained in enamel and dentine are calcium (Ca^{2+}) and phosphate (PO_4^{3-}), therefore it will form a hydroxyapatite crystal ($Ca_{10}(PO_4)_6(OH)_2$).[7] Besides, there are also another contained minerals in tooth such as Mg^{2+}, Zn^{2+}, Cu^{2+} and Se^{4+}.[5]

Acidic condition are able to demineralize the minerals contained in enamel and dentine.[8] The acid can be sourced from water consumed or derived from bacteria. Demineralization occurs when the pH of the solution on the enamel surface is below 5.5.[5.7] Continuous acid condition can make enamel dissolve slowly and make cavity in the tooth.[5] the tooth that have been demineralized by acids are more susceptible to erosion and difficult to repair. The tooth that have been demineralized by acid are also more susceptible to caries.[7]

One of mineral that can be released when the tooth are exposed to acid are copper ions (Cu^{2+}). Copper ions have a very important role in the process of demineralization and remineralization of teeth. Copper ions can reduce the solubility of enamel in an acidic environment by making a protective layer in the form of copper phosphate ($Cu_3(PO_4)_2$), which prevents demineralization by stabilizing hydroxyapatite crystals on the enamel surface thereby make enamel resistant to erosion and caries. Copper ions are also able to become cariostatic agents by inhibiting the development and metabolism of bacteria.[9] Aside from copper ions, other minerals that can be released when the teeth are exposed to acids are selenium ions (Se^{4+}). The presence of selenium ions in tooth can increase the susceptibility of teeth to caries.[10]

Peat water is thought to cause more release of copper ions and less selenium ions than teeth. Based on the background above, there has not been any study regarding the effect of tooth immersed in peat water on the release of copper and selenium ions, thus such study should be conducted.

METHODS AND MATERIALS

This study received ethical clearance issued by the Ethical Committee, Faculty of Dentistry, Lambung Mangkurat University No. 129/KEPKG FKGULM/EC/2019. The method used in this study was true experimental with post test only with control group design which was divided into 3 groups. The tooth specimens were collected using simple random sampling. The first group acted as control group, which involved teeth immersed in artificial saliva. The second group involved teeth immersed in water from Water Utilities (PDAM) and the third group involved teeth immersed in peat water.

Tooth Specimen Preparation

The teeth used in this study were the maxillary left or right, first or second human premolars that were already extracted. The teeth were free from caries, fracture, or chipping. The number of teeth in each group was 6. Firstly, the teeth were placed in the oven for 2 days using laboratory oven at 100^{o}C. Afterwards, the teeth were cut between the crown and the root, i.e. in the CEJ (cementoenamel junction). The next step was to cut the crown into two equal pieces, crosswise between buccal and palatal side. One of the crown parts was taken for immersion according to treatment group and the other part was tested for copper and selenium ion levels. The dentin and pulp parts of the teeth were stained with nail polish with the intention to protect from immersion to prevent any effect on test results.

Immersion Procedure

After layering the dentin and pulp using nail polish, then the crowns were immersed for 7 days according to treatment group. Each group was immersed in 50 ml of liquid.

Pulverization Procedure

The immersed crowns were then pulverized using metal mortar and pestle. The crowns were pulverized until smooth and homogenous.

Specimen Preparation Procedure

Specimen preparation procedure began by mixing pulverized crowns with 50 ml bidistilled water. Afterwards, 5 ml of thick HNO_3 then added and heated on electric heater until the volume reduced to 15-20 ml. If the destruction is not yet completed and the solution is not clear, then another 5 ml of thick HNO_3 was added than reheated.

Copper Ion Level Analysis

Copper ions were determined using UV-Vis T80+ spectrophotometry (PG Instruments, United Kingdom) according to redox reaction between methylene ($C_{16}H_{18}ClN_3S$) and ascorbic acid ($C_6H_8O_6$) catalyzed by copper (II) with maximum wavelength of 666 nm. The prepared solution was diluted with citric acid ($C_6H_8O_7$) and disodium phosphate (Na_2HPO_4).

Afterwards, ascorbic acid and methylene blue were added, thus the liquid turned to blue and inserted into UV-Vis T80+ spectrophotometer cuvette for copper ion level analysis. The analysis was replicated two times.

Selenium Ion Level Analysis

Selenium ions were determined using UV-Vis T80+ spectrophotometry (PG Instruments, United Kingdom) based on the reaction between selenium and potassium iodite (KIO_2) in acid. This will create a complex with starch and a maximum wavelength of 570 nm. The prepared solution was added with NaOH and EDTA and centrifuged. The next step included addition of potassium iodite and starch to the solution, thus changed its color to yellow. Then, the solution was poured into UV-Vis T80+ spectrophotometer cuvette for selenium ion level analysis. The analysis was replicated two times.

The obtained data were evaluated statistically using SPSS 25.0. Data normality was tested using Shapiro-Wilk and homogeneity test using Levene's test. Parametric analysis was performed using One Way ANOVA with confidence interval of 95% (α = 0.05), followed by Post-hoc Dunnett's T3 to determine the significance value.

RESULTS

The result of copper and selenium ion release was obtained from the mean difference between treated and non-treated crown parts. The results of average release of copper and selenium ions can be seen in Figure 1 and 2.

Figure 1. The Mean Value and Standard Deviation of Released Copper Ions

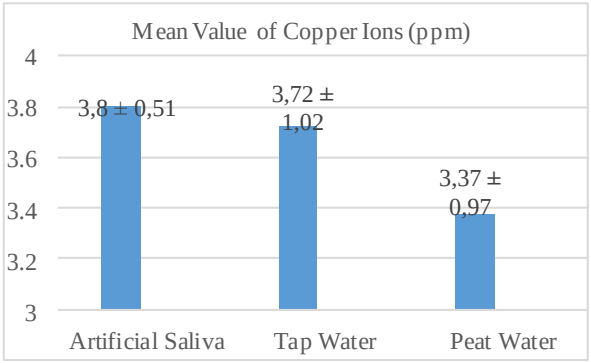

Figure 1 shows that the highest mean value of released copper ions is from the artificial saliva immersion group (3.8 ± 0.51 ppm), followed by the tap water immersion group (3.72 ± 1.02) and lastly from the peat water immersion group (3.37 ± 0.97 ppm).

Figure 2. The Mean value and Standard deviation of Released Selenium Ions

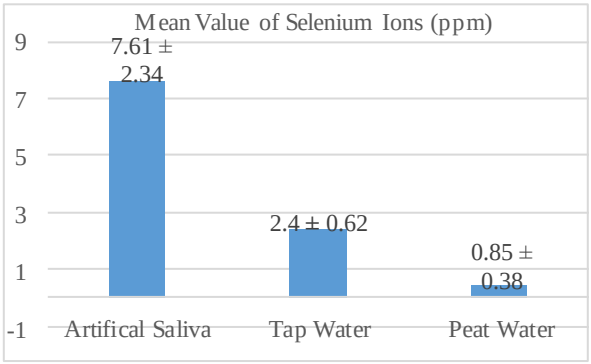

Figure 2 show that the highest mean value of released selenium ions is from the artificial saliva immersion group (7.61 ± 2.34 ppm), followed by the tap water immersion group (2.4 + 0.62 ppm), and lastly from the peat water immersion group (0.85 ± 0.38 ppm). The results of Post hoc Dunnett's T3 test shows a significant difference ($p<0.05$) between each immersion group, which can be seen in Table 1.

Table 1. The Results of Dunnet's T3 Test on Released Selenium Ions

	Artificial Saliva	Tap Water	Peat Water
Artificial Saliva	-	0,006*	0,002*
Tap Water	-	-	0,002*
Peat Water	-	-	-

** = significant difference ($p<0.05$)*

According to Table 1, there is a significant difference between the artificial saliva immersion group and the tap water immersion group. The artifical saliva immersion group and the peat water immersion group also have a significant difference. The significant difference also can be seen between the tap water immersion group and the peat water immersion group.

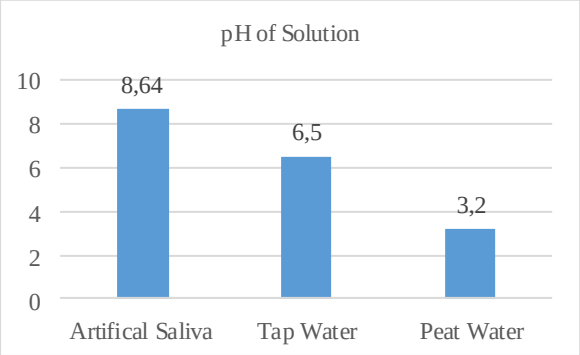

Figure 3. pH of Solution

Figure 3 shows that the pH of each immersion solution group is 8.64 for artifical saliva, 6.5 for tap water and 3.2 for peat water.

Figure 4. Copper Ions Content and Selenium Ions Content (ppm) in Each Immersion Solutions

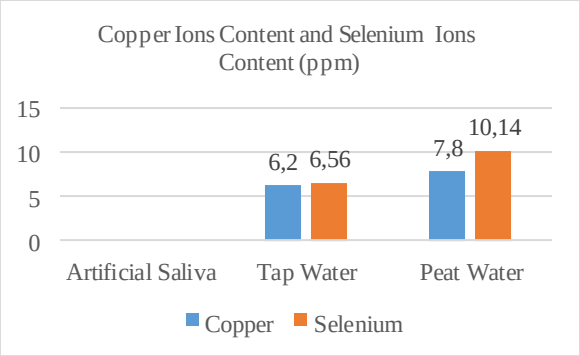

Figure 4 shows that artifical saliva solution does not contain copper and selenium ions. Tap water contains 6.2 ppm copper ions and 6.56 ppm selenium ions. Peat water contains 7.8 ppm copper ions and 10.14 ppm selenium ions.

DISCUSSION

There are several factors that prevent demineralization in teeth. One of the factors that affects tooth demineralization is solution acidity or H^+ content in a solution. When H^+ contacted hydroxyapatite crystals, then H^+ will create a bond with phosphate ions (PO_4^{3-}) on hydroxyapatite crystals, thus forming $H_2PO_4^-$ or phosphoric acid.[11] Other factors that affected the release of ions in hydroxyapatite crystals include the formation of chelate complex between acid and minerals in hydroxyapatites. Carboxylic acid with COOH group will dissociated in the solution, thus forming H^+ that bonds with H_2O to form H_3O^+ and the remaining COO^- will create a chelation with calcium ions (Ca^{2+}). H^+ ions in the solution will attack hydroxyapatite crystals, contrary to two COO^- anions that formed will create a bond with three calcium ions in hydroxyapatite, forming chelation and pull the calcium ion out of hydroxyapatite crystals. The stronger the bond, the higher the calcium ions pulled out from hydroxyapatite, followed by the release of other ions.[7]

Theoretically, acidic water or water with low pH causes the release of ions such as copper contained in teeth. However, there are several things that make these ions, especially copper, more resistant to demineralization from acid attack or environmental changes. One of the factors that may cause insignificant difference between immersion groups and prevent the release of copper ions was due to higher charge density in copper ions compared to calcium ions in hydroxyapatite crystal, thus made it stronger to acid attack.[12,13] Copper ions in hydroxyapatite crystals were formed by adsorption process that occurs between hydroxyapatites and copper ions. Copper will be adsorbed by hydroxyapatite by substituting calcium ions with few copper ions through diffusion process on hydroxyapatite crystal lattice, thus forming a reaction as follows:

$$Ca_{10}(PO_4)_6(OH)_2 + xCu^{2+} \rightarrow (Ca_{10-x})Cu_x(PO_4)_6(OH)_2 + xCa^{2+}$$

In above substitution reaction, the substitution between calcium and copper occurred because the size of ion radius between both ions were almost equal, in which calcium ions with 0.099 nm and copper ions with smaller radius of 0.072 nm. Even though the radius of copper ion was smaller that may aid in demineralization, copper ion had higher charge density, thus more stable on the hydroxyapatite crystals, thus making it resistant to demineralization.[12,13]

Other factor that may affect copper ions is that the copper ions contained in teeth created a protective layer in the form of copper (II) phosphate ($Cu_3(PO_4)_2$) on the enamel surface, thus making the enamel more resistant to environmental changes, especially acidic environment.[9]

The results of this study showed that copper ion release on peat water group was less than immersion in artificial saliva and tap water. This may be caused by adsorption reaction between hydroxyapatites and free copper ions in the solution.[14]

Copper ions can only be maximally adsorbed by hydroxyapatites on pH 7.9, while above that, the adsorption process is minimal, or even none.[15] Acidic pH (3.2) on peat water caused a more effective adsorption process of copper ions by hydroxyapatite crystals compared to artificial saliva (pH 8.64) and tap water (pH 6.5).[16]

Higher copper ion content in peat water (7.8 ppm) compared to tap water (6.2 ppm) also caused higher adsorption of copper ions in peat water compared to tap water. Copper ions contained in tap water became a factor that caused less copper ion release compared to artificial saliva with no copper ion content.[13,17]

Similar to copper, selenium ions were also less released in peat water. This may be due to pKa of peat water that was not as low as its pH. The term pKa describes acid strength. Peat water, mostly contained humic substances, only had 4.96 pKa, thus ions in hydroxyapatite crystals, especially selenium, is not released much even though peat water had pH of 3.2.[7,18] This was supported by the many content of organic substances in peat water. It is known that selenium ions contained in hydroxyapatite is obtained from adsorption process that occurs in hydroxyapatite crystal by substituting between selenium ions and phosphor (P^{5+}) which can be seen in the reaction as follows:

$$Ca_{10}(PO_4)_6(OH)_2 + _{0,25}SeO_4^{2-} \longrightarrow Ca_{9,75}(PO_4)_{5,75}(SeO_4)_{0,25}(OH)_{1,75}$$

Smaller radius of selenium ions, i.e. 0.035 nm, compared to phosphor ions with 0.05 nm causes selenium ions to be more unstable, thus more easily demineralized by acid attack.[12] However, the organic substances in peat water can stabilized the bond with selenium ions contained in hydroxyapatite crystals, thus made it difficult to demineralized by acid.[19]

The result showed that there was a significant difference between immersion in artificial saliva and tap water and peat water. This may occur due to adsorption reaction of selenium ions in the form of selenate (SeO_4^{2-}) by hydroxyapatite crystals in the enamel with the mechanism of bond complex formation on the surface of hydroxyapatites. The surface of hydroxyapatite crystals becomes positive in acidic solution, thus increasing the adsorption capacity of selenium through ligand exchange. Adsorption reaction of selenium ions can only occur under pH 7.86, whilst above it, the adsorption reaction is second to none.[20]

Hydroxyapatite crystals have positive charge in pH under 7.86, thus enabling the crystal to adsorb selenium ions in the form of free selenate with negative charge in acidic solution, especially peat water. Contrary to solution condition with pH above 7.86 or alkaline, selenium ion adsorption by hydroxyapatite crystals decreased sharply due to adsorption competition between selenate and OH^- ions found in abundance in alkaline solution, whereas selenate and OH^- ions both have negative charge. The pH of artificial saliva was 8.64, while pH of tap water was 6.5 and peat water was 3.2. This fact causes less and even no adsorption reaction of selenium ions by hydroxyapatite crystals in artificial saliva compared to tap and peat water.[20,21] Other theory also stated that selenium ion adsorption only occur on pH under 6.5, which means that there are more selenium ion adsorption in peat water compared to tap water and the difference between both groups was significant.[20]

Other factor that causes differences between treatment groups was different selenium ion content between the two groups. Selenium ion content in peat water (10.14 ppm) was more than tap water (6.56 ppm), thus produced more adsorption by hydroxyapatite crystals in peat water immersion. Artificial saliva does not have selenium ions, which means that adsorption by hydroxyapatite crystals cannot occur compared to the other 2 groups.[17,22]

Theoretically, selenium ions in teeth can increase tooth vulnerability to demineralization process. However, peat water with a lot of organic substances that can stabilized the bond between selenium ions and hydroxyapatite crystals, thus may decrease demineralization process.[19,23] This was contradictive with demineralization process caused by caries. Selenium ion content in teeth can increase demineralization process caused by caries because there is no organic substance in caries, thus selenium ion is more vulnerable to demineralization.[24] Selenium ion is also known to decrease fluoride uptake in hydroxyapatite crystals.[25]

The results of this study showed that copper ions in teeth was not affected by peat water, thus copper ion release is not the factor of high teeth damage in South Kalimantan. Contrary to copper ions, only a few amounts of selenium ions released were affected by peat water. This showed that selenium ions were found in abundance in the teeth of the population of South Kalimantan, thus contributed to high factor of teeth damage in South Kalimantan. In conclusion, there was no effect of teeth immersed in peat water on copper ion release, but there was an effect on selenium ion release.

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