

# Dental Erosion by Beverages and Determination of Trace Elements in Teeth by Atomic Absorption Spectrometry

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Received 19 June 2016; accepted 16 July 2016; published 19 July 2016

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## Abstract

The analysis of trace elements in human tooth for use as biomarkers continues to generate considerable interest in environmental and bioanalytical studies, medical diagnostic and forensic science. This study investigated concentrations of essential and toxic elements (Zn, Cu, Pb, Ni, Co and Cd) using flame atomic absorption spectroscopy (FAAS) and (Na and K) flame atomic emission spectroscopy (FAES) in human tooth obtained from Misan province, Iraq. The influence of smoking habits and born children on the levels of trace elements in additional to dental erosion by some beverages in the tooth samples were investigated. The overall of mean concentrations of (Zn 185.2 ppm, Cu 36.11 ppm, Pb 18.63 ppm, Ni 13.44 ppm, Co 3.45 ppm, and Cd 0.58 ppm, Na 11.683 ppm and K 115.27 ppm), (Zn 178.243 ppm, Cu 35.877 ppm, Pb 15.64 ppm, Ni 12.89 ppm, Co 3.421 ppm, Cd 0.455 ppm, Na 11,558.04 ppm and K 114.04 ppm) in smokers and non-smokers tooth groups respectively, (Zn 81.12 ppm, Cu 24.37 ppm, Pb 11.18 ppm, Ni 13.68 ppm, Co 3.32 ppm, Cd 0.44 ppm, Na 8227 ppm and K 76.42 ppm), (Zn 80.58 ppm, Cu 24.30 ppm, Pb 10.86 ppm, Ni 12.74 ppm, Co 2.71 ppm, Cd 0.23 ppm, Na 8165 ppm and K 72.80 ppm) in mothers and wives tooth groups respectively. However the w/w% loss of tooth immersed in following order of beverages (green tea, black tea, cinnamon, hibiscus, vinegar and citrus aurantifolia (9.09, 9.68, 12.36, 15.01, 16.77 and 17.38), (8.19, 8.25, 9.76, 13.37, 14.02 and 15.33) in smokers and non-smokers groups respectively, (11.25, 11.47, 13.30, 15.75, 19.58, and 20.91), (8.31, 7.96, 11.39, 13.49, 17.40, and 17.67) in mothers and wives groups respectively. However, there is a statistically significant variance found Zn ( $t_{cal}$  5.029,  $t_{tab}$  2.131), Pb ( $t_{cal}$  4.8538,  $t_{tab}$  2.131), Ni ( $t_{cal}$  5.243,  $t_{tab}$  2.131), Cd ( $t_{cal}$  3.9062,  $t_{tab}$  2.131) and Na ( $t_{cal}$  3.0832,  $t_{tab}$  2.131) between smokers and non-smokers tooth groups. However, the Co ( $t_{cal}$  2.7403,  $t_{tab}$  2.131) also shows statistically significant variance between mothers and wives groups. There is no statistically significant variance in w/w% loss after immersed the tooth in some beverages but in general it was higher in smokers than non-smokers and in mothers higher than wives.

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## Keywords

**Flame Atomic Absorption Spectroscopy, Flame Atomic Emission Spectroscopy, Trace Element Concentrations, Biomarker**

## 1. Introduction

Biomonitoring of trace elements in human teeth has become an important tool to evaluate an individual's nutritional and environmental status [1]-[5]. Trace elements can be ingested by humans via different routes, including ingestion in the food and water or by deliberate consumption of soil and by dermal absorption. The presence and/or absence of trace elements in the environment influences their availability to humans [1]. For example, in the rural areas of Finland, the concentrations of Zn and Mg found in human dentine correlate with their concentrations in the soil [6], and dental fluorosis has been linked to the concentrations of fluoride F and Pb present in the drinking water [1] [4]. The concentration of Pb in the teeth can be used as an index of environmental pollution [7]. This element is preferentially incorporated and stored in calcified tissues, such as the teeth [8]. Pb concentration above 4 mg/kg in teeth has been suggested as being indicative of Pb toxicity [5] [9].

Sources of Cd include emissions from fuel combustion, tobacco smoke, phosphate fertilizer, sewage sludge, metal smelting, and disposal of metal waste. Other sources include the use of Cd for industrial applications, such as in the production of pigments, stabilizers, and alloys. In addition, Cd is present in trace amounts in certain foods, such as leafy vegetables, potatoes, grains and seeds, liver and kidney, and crustaceans and molluscs. Exposure to environmental Cd has been linked with an increased risk of dental caries [10]. Other studies have demonstrated that nutritional deficiencies can be detected by analyzing the chemical composition of teeth and that such deficiencies can affect teeth during dentition, which is a critical growth period for teeth [6]-[8] [11]-[14]. Differences in the availability of foods and food choices, which are often influenced by social and cultural practices, may be important factors in determining which trace elements are ingested by humans and can complicate any attempts at changing dietary habits to alter the intake of trace elements [14].

The extracted healthy permanent molars teeth in total were collected due to periodontal disease or orthodontic treatment between 18 and 50 of age, 64 teeth for trace elements analysis, and 72 teeth for beverages erosion, by private dental clinics in Misan province, 16.072 Km<sup>2</sup> in area, with a population 1,412,234. All samples were divided into four groups, according to the men smoking habits and women got children. The principal aim of this study was to investigate the trace elements content in the groups. In additional to monitor the influence of following beverages, green tea (pH = 6.2), black tea (pH = 5.5), cinnamon (pH = 5.4), hibiscus (pH = 2.8), vinegar (pH = 2.4), and citrus aurantifolia (pH = 2.1) on the dental erosion.

## 2. Materials and Methods

### 2.1. Instrumentation

Aurora atomic absorption spectrophotometer model (AI-1200) was used with an air-acetylene burner (slot length 11 cm). Instrument setting were: lamp current, 10 mA, slit width, 0.2 nm, air flow, 1.8 L/min, ignition flow 2.4 L/min. These conditions were maintained constant throughout the measurements and Jenway PFP7 flame photometer was used with LPG liquid petroleum gas for sodium and potassium determination. The operating conditions adjusted according to the standard guidelines of the manufacturers. The quantitative determinations of the elements in samples were done by using calibration curves which obtained from diluted stock standard elements 1000 mg/L. The concentrations of the analytes were obtained directly from the FAAS, but through the linear regression's equations after the correction of the absorbance signal from the blank as shown in **Table 1**.

### 2.2. Reagents

A standard solution of each element was prepared immediately by dilution of 1000 mg/L stock solution (sigma aldrich) prior to use. Deionized water was used throughout this experiment. All solvents and reagents such as HNO<sub>3</sub>, H<sub>2</sub>O<sub>2</sub> were analytical reagent (AnalaR) grade.

**Table 1.** Operating parameters of FAAS, wavelength, slit width, (HCL) current, flame and limit of detection (LOD) for each element.

Parameters	Elements					
	Cu	Zn	Co	Pb	Ni	Cd
Wave length (nm)	324.7	213.9	240.7	217.0	232.0	228.8
Slit width (nm)	0.2	0.2	0.2	0.2	0.2	0.2
HCL amp current (mA)	6	5	7	5	7	5
Flame	Air/acetylene	Air/acetylene	Air/acetylene	Air/acetylene	Air/acetylene	Air/acetylene
LOD (ppm)	0.03	0.03	0.05	0.03	0.03	0.01

### 2.3. Samples Collection

The teeth were collected from private dental clinics in Misan province. 64 healthy extracted molar teeth for trace elements analysis, 32 males teeth, 16 smokers group and 16 non-smokers, in contrast 32 female's teeth, 16 mothers teeth group and 16 wives teeth group. Furthermore 72 healthy molar teeth for immersion in beverages, three teeth from each group as classified above, immersed in each single beverage, 18 teeth from each group were used, and all teeth were labeled in dry cellophane bags.

### 2.4. Preparation the Tooth Samples

To prevent sample contamination, all laboratory glassware was cleaned with detergent solution then rinsed with 10% nitric acid, then rinsed with deionized water before use. Each tooth was soaked in a solution 10% (v/v) hydrogen peroxide for 1 hour to remove any organic material, rinsed with deionized water, air dried, and weighed, some kept for immersion in beverages. The others teeth, each tooth digested with Aristar reagent for trace elements analysis, using 4 ml of 70% (v/v) nitric acid and 3 ml 70% (v/v) perchloric acid in a conical flask, heated on a hotplate in fume hood until a transparent liquid was produced which adjusted to 10 ml with deionized water.

### 2.5. Preparation the Beverages

The commercially brewing drinks, prepared by grinding the dry clean (leaves, fruit, flowers, and cortex) by ceramic mortar and pistol, sieving by a sieve mesh, added to 150 ml deionized water in a 250 ml beaker, filtered by a filter paper, collected in a conical flask 150 ml, the pH of the solutions monitored by the pH meter.

### 2.6. Teeth Immersion

Three dry molar teeth from each group were immersed in each single beverage for (3, 6, 9, 12, and 15 days) respectively, with a slow shaking by a shaker during immersion, the teeth were dried on air and weight after each period time, the mean weight calculated.

### 2.7. Stock Solutions for Trace Element Analysis

Working range standard solutions used to construct calibration curves for each element were prepared by serial dilution of 1000 ppm standard stock solutions of each element. The calibration curve for each element was constructed by plotting the absorbance obtained from FAAS analysis of the standard solution versus the element concentration in ppm. Each sample was analyzed in triplicate and the average of trace element concentrations in the tooth samples were calculated using the constructed calibration curves.

## 3. Results and Discussion

### 3.1. Calibration Curves

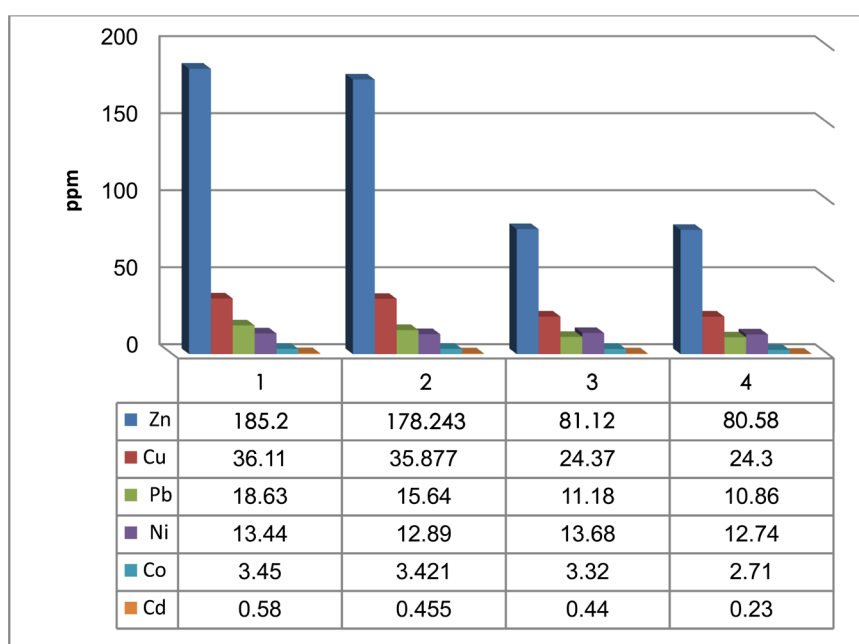
The trace elements concentrations in ppm obtained from the constructed calibration curves for the investigated trace elements in the tooth samples. **Table 1** indicates the wavelength used for AAS elemental analysis, the limits of detection LOD which is defined as the minimum detectable amount of metal, was calculated using the equation:  $LOD = 3 s/m$ ; where  $s$  is the signal of the blank and  $m$  is the slope of the calibration curve [15]. The trace

of the element concentration varied widely in the tooth samples as demonstrated by the large range of trace element concentration.

### 3.2. Trace Elements Concentration of Smokers and Non-Smokers Teeth Groups

A summary of the levels of trace elements concentrations found in the Teeth of smokers and non-smokers are shown in **Figure 1**. The order of the mean trace elements concentrations in tooth samples of smokers group is Zn > Cu > Pb > Ni > Co > Cd. Overall mean concentrations of Zn (185.2 ppm), Cu (36.11 ppm), Pb (18.63 ppm), Ni (13.44 ppm), Co (3.45 ppm) Cd (0.58) in smokers, in contrast, corresponding mean levels of Zn (178.24 ppm), Cu (35.87 ppm), Pb (15.64 ppm), Ni (12.89 ppm), Co (3.42 ppm) and Cd (0.45 ppm) were obtained in non smokers. To evaluate the differences between the mean trace element concentrations obtained in smokers and non-smokers, the mean trace elements concentration obtained for the two categories were subjected to a statistical student t-test analysis.

At a 95% confidence level, there were significant differences between the mean concentration of Zn ( $t_{cal}$  5.029,  $t_{tab}$  2.131), Cu ( $t_{cal}$  2.056,  $t_{tab}$  2.131) Pb ( $t_{cal}$  4.8538,  $t_{tab}$  2.131), Ni ( $t_{cal}$  5.243,  $t_{tab}$  2.131), Co ( $t_{cal}$  1.990,  $t_{tab}$  2.131), and Cd ( $t_{cal}$  3.9062,  $t_{tab}$  2.131) obtained in smokers and non-smokers tooth groups respectively, as shown in **Table 2**.



**Figure 1.** Concentrations of trace elements in ppm 1. smokers, 2. Non smokers, 3. Mothers and 4. Wives, healthy teeth groups, (n = 16).

**Table 2.** Concentrations of trace elements (ppm) in smokers, non-smokers, mothers and wives, in healthy teeth groups, (n = 16).

Elements	Smokers Mean $\pm$ SD	Non Smokers Mean $\pm$ SD	t-value	Mothers Mean $\pm$ SD	Wives Mean $\pm$ SD	t-value
Cd	0.58 $\pm$ 0.10	0.45 $\pm$ 0.08	3.9062	0.44 $\pm$ 0.85	0.23 $\pm$ 0.31	0.9284
Co	3.45 $\pm$ 0.05	3.42 $\pm$ 0.03	1.9903	3.32 $\pm$ 0.51	2.71 $\pm$ 0.73	2.7403
Ni	13.44 $\pm$ 0.21	12.89 $\pm$ 0.03	5.2430	13.68 $\pm$ 2.75	12.74 $\pm$ 1.41	1.2166
Pb	18.63 $\pm$ 1.97	15.64 $\pm$ 1.48	4.8538	11.18 $\pm$ 0.61	10.86 $\pm$ 0.82	1.2524
Cu	36.11 $\pm$ 0.30	35.87 $\pm$ 0.34	2.0560	24.37 $\pm$ 0.12	24.30 $\pm$ 0.05	2.1538
Zn	185.20 $\pm$ 4.25	178.24 $\pm$ 3.55	5.0290	81.12 $\pm$ 0.93	80.58 $\pm$ 0.54	2.0089
K	115.27 $\pm$ 1.30	114.04 $\pm$ 2.10	1.9922	76.42 $\pm$ 3.10	72.80 $\pm$ 7.76	1.7328
Na	11683 $\pm$ 120	11,558.04 $\pm$ 109	3.0832	8227 $\pm$ 70	8165 $\pm$ 98	2.0592

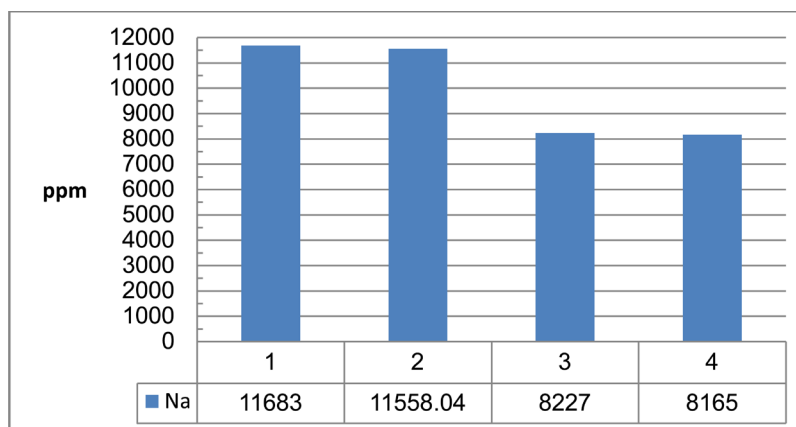
### 3.3. Trace Element Concentrations of Mothers and Wives Teeth Groups

Generally, **Figure 1** shows the mean levels of the most trace elements were not significantly higher in mothers compared to wives. The mean levels of Zn (81.12 ppm), Cu (24.37 ppm), Ni (13.68 ppm), Pb (11.18 ppm), Co (3.32 ppm) and Cd (0.44 ppm) respectively obtained in mothers. Interestingly the mean levels of Zn (80.58 ppm), Cu (24.30 ppm), Ni (12.74 ppm), Pb (10.86 ppm), Co (2.71 ppm) and Cd (0.23 ppm) respectively obtained in wives. The concentration of Co obtained in mothers was significantly and statistically different ( $t > 2.131$ ) from the corresponding level in wives. At the 95% confidence level, there were no significant differences ( $t < 2.131$ ) between the trace element concentrations obtained between mothers and wives.

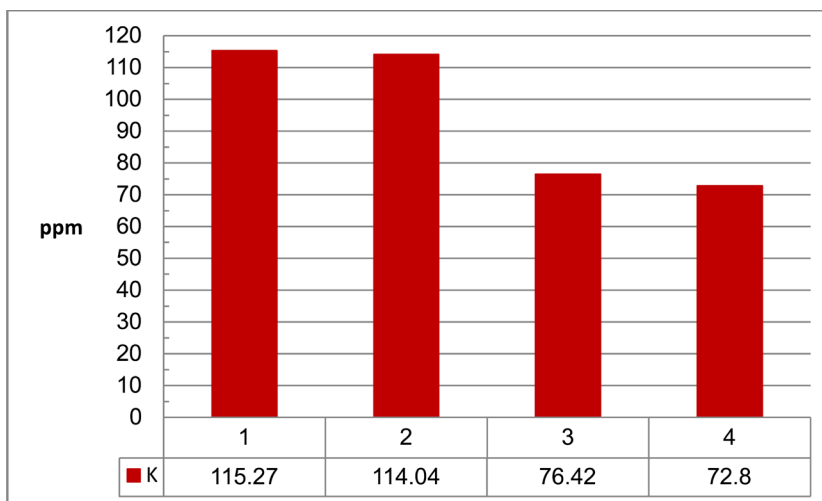
The concentration of Zn ( $t_{cal}$  2.0089,  $t_{tab}$  2.131), Cu ( $t_{cal}$  2.1538,  $t_{tab}$  2.131) Pb ( $t_{cal}$  1.2524,  $t_{tab}$  2.131), Ni ( $t_{cal}$  1.2166,  $t_{tab}$  2.131), Co ( $t_{cal}$  2.7403,  $t_{tab}$  2.131), Cd ( $t_{cal}$  0.9284,  $t_{tab}$  2.131) obtained in mothers and wives, as shown in **Table 2**.

The order of the mean concentrations of sodium and potassium in tooth samples of smokers group is Na > K, the mean levels of Na (11,683 ppm), K (115.27 ppm) respectively obtained in smokers and Na (11,558.04 ppm), K (114.04 ppm) in non-smokers, as shown in **Figure 2** and **Figure 3**. The concentration of Na ( $t_{cal}$  3.0832,  $t_{tab}$  2.131), K ( $t_{cal}$  1.9922,  $t_{tab}$  2.131) obtained in smokers and non-smokers so, the difference mean concentrations of Na between smokers and non smokers were significant as shown in **Table 2**.

On the other hand the mean concentration of sodium and potassium in tooth samples of mothers group is following the same previous order Na > K, the mean level of Na (8227 ppm), K (76.42 ppm), and Na (8165 ppm), K (72.80 ppm) respectively in wives group as shown in **Figure 2** and **Figure 3**. There are no significant differ-



**Figure 2.** Concentrations of sodium in ppm 1. smokers, 2. non smokers, 3. mothers and 4. wives, healthy teeth groups (n = 16).



**Figure 3.** Concentrations of potassium in ppm 1. smokers, 2. non smokers, 3. mothers and 4. wives, healthy teeth groups.

ences between concentrations of Na ( $t_{cal}$  2.0592,  $t_{tab}$  2.131) and K ( $t_{cal}$  1.7328,  $t_{tab}$  2.131) obtained in mothers and wives, as shown in **Table 2**.

### 3.4. Effect of Beverages on the Dental Erosion of Smokers and Non-Smokers Teeth Groups

The second part of the study was to determine the effect of some beverages on weight loss percentage of the smokers, non-smokers, mothers and wives tooth groups as shown in **Table 3**. The influence of the following beverages green tea (pH = 6.2), black tea (pH = 5.5), cinnamon (pH = 5.4), hibiscus (pH = 2.8), vinegar (pH = 2.4) and citrus aurantifolia (pH = 2.1) on smokers, non-smokers, mothers and wives tooth groups.

**Table 3** shows the influence of the beverages on the average weight loss percentage of smokers and non-smokers tooth groups, the influence of beverages on smokers and non-smokers groups following the order green tea (pH = 6.2) < black tea (pH = 5.5) < cinnamon (pH = 5.4) < hibiscus (pH = 2.8) < vinegar (pH = 2.4) < and citrus aurantifolia (pH = 2.1), the average weight loss percentage of the smokers tooth, green tea (pH = 6.2) w/w% (9.09), black tea (pH = 5.5) w/w% (9.68), cinnamon (pH = 5.4) w/w% (12.36), hibiscus (pH = 2.8) w/w% (15.01), vinegar (pH = 2.4) w/w% (16.77) and citrus aurantifolia (pH = 2.1) w/w% (17.38) on the other hand for non-smokers green tea (pH = 6.2) w/w% (8.19), black tea (pH = 5.5) w/w% (8.25), cinnamon (pH = 5.4) w/w% (9.76), hibiscus (pH = 2.8) w/w% (13.37), vinegar (pH = 2.4) w/w% (14.02) and citrus aurantifolia (pH = 2.1) w/w% (15.33) as shown in **Figure 4**.

### 3.5. Effect of Beverages on Dental Erosion of Mothers and Wives Teeth Groups

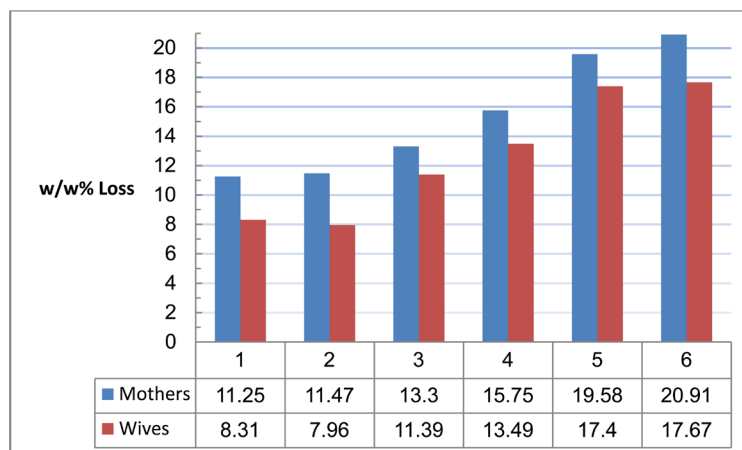
**Table 3** shows the influence of the beverages on the average weight loss percentage of mothers and wives tooth groups, the influence of beverages on mothers group following the order green tea (pH = 6.2) < black tea (pH = 5.5) < cinnamon (pH = 5.4) < hibiscus (pH = 2.8) < vinegar (pH = 2.4) < and citrus aurantifolia (pH = 2.1), but for wives group are green tea (pH = 6.2) > black tea (pH = 5.5) < cinnamon (pH = 5.4) < hibiscus (pH = 2.8) < vinegar (pH = 2.4) < and citrus aurantifolia (pH = 2.1). The average weight loss percentage of the mothers tooth group, green tea (pH = 6.2) w/w% (11.25), black tea (pH = 5.5) w/w% (11.47), cinnamon (pH = 5.4) w/w% (13.30), hibiscus (pH = 2.8) w/w% (15.75), vinegar (pH = 2.4) w/w% (19.58) and citrus aurantifolia (pH = 2.1)

**Table 3.** Mean w/w% loss of smokers, non-smokers, mothers, and wives teeth groups immersed in green tea, black tea, cinnamon, hibiscus, vinegar and citrus aurantifolia beverages (n = 3).

Beverages	pH	Smokers Mean ± SD	Non Smokers Mean ± SD	t-value	Mothers Mean ± SD	Wives Mean ± SD	t-value
Green tea	6.2	9.09 ± 5.64	8.19 ± 5.71	0.3957	11.25 ± 6.51	8.31 ± 5.49	0.2086
Black tea	5.5	9.68 ± 5.84	8.25 ± 5.36	0.3344	11.47 ± 6.24	7.96 ± 4.54	0.1466
Cinnamon	5.4	12.36 ± 6.81	9.76 ± 6.81	0.2646	13.30 ± 8.34	11.39 ± 6.98	0.3379
Hibiscus	2.8	15.01 ± 7.90	13.37 ± 7.64	0.3608	15.75 ± 7.86	13.49 ± 7.22	0.3084
Vinegar	2.4	16.77 ± 8.89	14.02 ± 8.68	0.2999	19.58 ± 11.16	17.40 ± 10.35	0.3661
Citrus aurantifolia	2.1	17.38 ± 9.05	15.33 ± 8.93	0.3506	20.91 ± 11.71	17.67 ± 10.61	0.3135



**Figure 4.** Mean w/w% loss of smokers and non-smokers healthy tooth groups, immersed in 1. green Tea, 2. black tea, 3. cinnamon, 4. hibiscus, 5. vinegar and 6. citrus aurantifolia, (n = 3).



**Figure 5.** The mean w/w% loss of mothers and wives healthy tooth groups, immersed in 1. green tea, 2. black tea, 3. cinnamon, 4. hibiscus 5. vinegar and 6. citrus aurantifolia (n = 3).

w/w% (20.91), but for wives group are green tea (pH = 6.2) w/w% (8.31), black tea (pH = 5.5) w/w% (7.96), cinnamon (pH = 5.4) w/w% (11.39), hibiscus (pH = 2.8) w/w% (13.49), vinegar (pH = 2.4) w/w% (17.40) and citrus aurantifolia (pH = 2.1) w/w% (17.67) as shown in **Figure 5**.

**Table 3** shows at the 95% confidence level, there were no significant differences ( $t < 4.303$ ) between the weight loss percentage of smokers and non-smokers tooth groups in following beverages, green tea (pH = 6.2) ( $t_{cal}$  0.3957,  $t_{tab}$  4.303), black tea (pH = 5.5) ( $t_{cal}$  0.3344,  $t_{tab}$  4.303), cinnamon (pH = 5.4) ( $t_{cal}$  0.2664,  $t_{tab}$  4.303), hibiscus (pH = 2.8) ( $t_{cal}$  0.3608,  $t_{tab}$  4.303), vinegar (pH = 2.4) ( $t_{cal}$  2.999,  $t_{tab}$  4.303), and citrus aurantifolia (pH = 2.1) ( $t_{cal}$  0.3506,  $t_{tab}$  4.303), also there were no significant differences ( $t < 4.303$ ) between the weight loss percentage of mothers and wives tooth groups in following beverages, green tea (pH = 6.2) ( $t_{cal}$  0.2086,  $t_{tab}$  4.303), black tea (pH = 5.5) ( $t_{cal}$  0.1466,  $t_{tab}$  4.303), cinnamon (pH = 5.4) ( $t_{cal}$  0.3379,  $t_{tab}$  4.303), hibiscus (pH = 2.8) ( $t_{cal}$  0.3084,  $t_{tab}$  4.303), vinegar (pH = 2.4) ( $t_{cal}$  0.3661,  $t_{tab}$  4.303), and citrus aurantifolia (pH = 2.1) ( $t_{cal}$  0.3135,  $t_{tab}$  4.303).

#### 4. Conclusions

In general, this study shows that the mean levels of trace elements are found higher in smokers group than non-smokers group. There are statistically significant differences concerning the elements Zn ( $t_{cal}$  4.871,  $t_{tab}$  2.131), Pb ( $t_{cal}$  4.989,  $t_{tab}$  2.131), Ni ( $t_{cal}$  5.111,  $t_{tab}$  2.131) and Cd ( $t_{cal}$  3.787,  $t_{tab}$  2.131) obtained in smokers and non-smokers.

On the other hand, we did not find any statistically differences in trace elements levels between mothers and wives groups except the Co ( $t_{cal}$  2.654,  $t_{tab}$  2.131), but in general the trace elements levels found higher in mothers group than wives group.

The result showed that the tooth erosion by different beverages depended on the pH of the solution, which increased with lower pH value, concerning to the effect of direct attack by the hydrogen ion on the teeth.

There are no statistically significant differences of beverages effect on the teeth erosion between smokers and non-smokers groups, mothers and wives groups; but the teeth erosion of smokers was higher than non-smokers and the teeth erosion of mothers group was also higher than wives.

#### Acknowledgements

The authors would like to acknowledgment the private dental clinics in Misan province for their efforts during collecting the tooth samples.

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