*Toxicology*

# LEVELS OF TRACE ELEMENTS IN COMMERCIAL FRUIT JUICES IN JEDDAH, SAUDI ARABIA

S.M. FARID*
M.A. ENANI*

*SUMMARY: Potable water, fruit juices and soft drinks are some of the most widespread beverages in the habitual diet, and they can contribute to trace element dietary intake. Heavy metals' toxicity is the result of their interactions with the enzymatic systems from the animal cells or some constituents of cells' membranes. Population can be contaminated with heavy metals by ingestion of contaminated or polluted food and water. The concentration of heavy metals in food products is varied, depending on their origin, storage conditions and processing technologies.*

*We determined the concentrations of zinc(Zn), copper(Cu), iron(Fe), chromium(Cr), manganese(Mn), cobalt(Co) and nickel(Ni) in apple, orange and mango fruit juice samples of 15 different brands widely consumed in Saudi Arabia. Graphite furnace atomic absorption spectrometry was used to analyze fruit juice samples processed with a $HNO_3$ - $V_2O_5$ acid digestion. In analyzed Samples, the mean (±SD) concentration values in ppb of Zn, Fe, Cu, Mn, Co, Cr, and Ni in apple juice are: (524.00±43.06); (325.36±23.12);(317.79±21.56); (23.48±2.23); (8.07±0.95); (6.36±0.94); and (6.21±0.90) respectively.*

*The mean (±SD) concentration values in ppb of Zn, Fe, Cu, Mn, Co, Cr, and Ni in orange juice are: (894.80±45.21); (361.27±24.11); (500.00±38.88); (20.93±2.36); (7.93±1.02);(5.93±0.92) and (5.73±0.91) respectively. Finally, the mean (±SD) concentration values in ppb of Zn, Fe, Cu, Mn, Co, Cr, and Ni in mango juice are: (486.57±35.25); (463.50±32.67); (461.07±29.49); (21.85±2.58); (8.14±1.28); (7.64±1.02) and (5.93±0.96) respectively. Our results for fruit juice are compared with Recommended Dietary Allowance (RDA) values and also with the corresponding values of different countries available in literature.*

*Key Words: Fruit juice, zinc, iron, copper, manganese, cobalt, chromium, nickel.*

## INTRODUCTION

Juices are the perfect fast food for today's eat-on-the-rum lifestyle. They contain all the goodness of the whole product in a condensed form. 100% juices are a convenient way for adults and children to get a part of their recommended 4.5 or more cups of fruits and vegetables each day. The 2005 Dietary Guidelines for Americans recommended consumption of several cups per day of fruits and vegetables, and acknowledge the role that 100% juices can play as part of this fruit and vegetable allowance. According to the new USDA My Pyramid food guidance program, there are portion sizes and recommended amounts of 100% juices for children and adults, depending on one's age, gender, and level of physical activity (1,2). 100% fruit juices are nutritious

*From Nuclear Engineering Department, King Abdulaziz University, P.O. Box 80204, Jeddah-21589, Saudi Arabia.

Table 1: Mean levels of trace elements in apple, orange and mango juice samples.

| Element | Apple Juice (ppb) | Orange Juice (ppb) | Mango Juice (ppb) | Mean (ppb) |
|---|---|---|---|---|
| Zn | 524.00 ± 43.06 | 894.80 ± 45.21 | 486.57 ± 35.25 | 635.05 ± 23.90 |
| Fe | 325.36 ± 23.12 | 361.27 ± 24.11 | 463.50 ± 32.67 | 383.38 ± 15.57 |
| Cu | 317.79 ± 21.56 | 500.00 ± 38.88 | 461.07 ± 29.49 | 426.28 ± 17.78 |
| Mn | 23.48 ± 2.23 | 20.93 ± 2.36 | 21.85 ± 2.58 | 22.08 ± 1.38 |
| Co | 8.07 ± 0.95 | 7.93 ± 1.02 | 8.14 ± 1.28 | 8.05 ± 0.63 |
| Cr | 6.36 ± 0.94 | 5.93 ± 0.92 | 7.64 ± 1.02 | 6.63 ± 0.55 |
| Ni | 6.21 ± 0.90 | 5.73 ± 0.91 | 5.93 ± 0.96 | 5.95 ± 0.53 |

beverages that have been enjoyed by adults and children for decades. 100% fruit juices can play an important role in a healthy diet because they offer great taste and a variety of nutrients found naturally in fruits (3). These juices are fat-free, nutrient-dense beverages that are rich in vitamins, minerals and naturally occurring phytonutrients that contribute to good health (3). In the present era of industrialization and development, one concern should be the health of the future generation. Children are the most vulnerable age group to any kind of contamination in the food chain. Majority of research confirms that 100% juice does not make children overweight (3).

Micronutrients are involved in numerous biochemical processes and an adequate intake of certain micronutrients relates to the prevention of deficiency diseases. Malnutrition is of major concern for many tropical developing countries. Iron (Fe) deficiency anemia, for example, affects one third of the world population. On the other hand, excessive iron intake has been associated with an overall increased risk of colorectal cancer (4).

Ingestion in food and beverages is likely to represent the principle route of chromium intake. Chromium (Cr) is a trace element, which has generated increased interest in recent years due to its essential character. Cr acts as a cofactor in maintaining the normal metabolism of glucose (5).

Manganese (Mn) is an essential metal that, at excessive levels in the brain, produces extra pyramidal symptoms similar to those in patients with Parkinson’s disease and decreased learning activity in school-aged children, and increased propensity for violence in adults (6).

Table 2 : Comparison of the elemental concentrations of Apple juice with the published values.

| Country | Concentration (µg/l) | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | Zn | Fe | Cu | Cr | Co | Mn | Ni | |
| Korea | | | | | 30 | | | 9 |
| Brazil | 691 | | 335.7 | | | | | 17 |
| Brazil | | | 416 | | | | | 17 |
| Spain | | | | 8 | | | | 5 |
| Iran | 560 | | | | | | | 20 |
| Nigeria | 474 | 416 | 535 | 10 | | 56 | 13 | 17 |
| France | | | | 16 | | | | 30 |
| USA | | 100-700 | | | | | | 19 |
| Saudi Arabia | 544.96 | 338.36 | 330.50 | 6.61 | 8.39 | 24.42 | 6.46 | Present Study |

Table 3: Comparison of the elemental concentrations of Orange juice with the published values.

| Country | Concentration (µg/l) | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | Zn | Fe | Cu | Cr | Co | Mn | Ni | |
| Spain | | | | 5.75 | | | | 5 , 12 |
| USA | 242-480 | 458-641 | 239-460 | | | | | 8 |
| Brazil | 842 | | 271 | | | | | 30 |
| Brazil | 511 | 760 | 309 | 11.5 | | 28.7 | | 15 |
| China | | | | | 10.3 | | | 31 |
| Korea | | | | | 20 | | | 9 |
| USA | 400 | | 400 | | | | | 19 |
| Malaysia | | 630 | | | | | 20 | 15 |
| Nigeria | | 800 | | 5.57 | | 16-90 | 19.7-58.7 | 15 |
| China | | 500 | | | | 93 | 11.3 | 15 |
| Australia | 350.2 | 628.3 | 370.8 | | 3.9 | 19.6 | 30 | 10 |
| Brazil | 731.5 | | 422.3 | 2.4 | 2.8 | 27.5 | 28.3 | 30 |
| Saudi Arabia | 921.64 | 372.11 | 515.00 | 6.11 | 8.17 | 21.56 | 5.90 | Present Study |

Both zinc (Zn) and copper (Cu), two essential trace minerals, perform important biochemical functions and are necessary for maintaining health throughout life. Zn constitutes about 33 ppm of adult body weight and is essential as a constituent of many enzymes involved in a number of physiological functions, such as protein synthesis and energy metabolism. Zn deficiency, resulting from poor diet, alcoholism and malabsorption, causes dwarfism, hypogonadism and dermatistis, while toxicity of Zn, due to excessive intake, may lead to electrolyte imbalance, nausea, anemia and lethargy (7). The adult human body contains about 1.5 - 2.0 ppm of Cu which is essential as a constituent of some metalloenymes and is required in haemoglobin synthesis and in the catalysis of metabolic oxidation. Symptoms of Cu deficiency in humans include bone demineralization, depressed growth, depigmentation, and gastro-intestinal disturbances, among others, while toxicity due to excessive intake has been reported to cause liver cirrhosis, dermatitis and neurological disorders (8).

Cobalt (Co) is essential in trace amounts for human life. It is part of vitamin B-12, and plays a key role in the body's synthesis of this essential vitamin. Cobalt is a necessary cofactor for making the thyroid hormone thyroxine. Cobalt has also been used as a treatment for anemia, because it causes red blood cells to be produced. The toxicity of cobalt is quite low compared to many other metals in soil (9). Exposure to very high levels of cobalt can cause health effects. Effects on the lungs, including asthma, pneumonia, and wheezing, have been found in workers who breathed high levels of cobalt (6,7).

In 1960s, some breweries added cobalt to beer to stabilize the foam. Some people who drank large quantities of the beer experienced nausea, vomiting, and serious effects on the heart. However, effects on the heart have not been seen in people with anemia or pregnant women medically treated with cobalt. Animal studies have found problems with the development of the fetus in animals exposed to high concentrations of cobalt during pregnancy. However, cobalt is also essential for the growth and development of certain animals (6,7).

The International Agency for Research on cancer has determined that cobalt is a possible carcinogen to humans. Studies in animals have shown that cobalt

Table 4: Comparison of the elemental concentrations of Mango juice with the published values.

| Country | Concentration (µg/l) | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | Zn | Fe | Cu | Cr | Co | Mn | Ni | |
| Venezuela | 339.2 | 776.5 | | | | 25.2 | | 32 |
| Pakistan | 300-700 | 63-230 | 500-700 | 7-25 | | 80 | 31.3 | 33 |
| United Arab Emirates (UAE) | | 590 | | | | 57 | 80 | 15 |
| Brazil | | | 530 | | | | | 30 |
| Saudi Arabia | 515.76 | 491.31 | 488.73 | 8.10 | 8.63 | 23.16 | 6.28 | Present Study |

causes cancer when placed directly into the muscle or under the skin. Cobalt did not cause cancer in animals that were exposed to it in the air, in food, or in drinking water. Cobalt and cancer studies on people are inconclusive (6,7).

Nickel (Ni) is an essential trace element. Human exposure to Ni may occur in industrial environment or through food chain. Ni plays some important role in biological systems such as in enzyme activity in hormonal control and also in RNA, DNA, and protein structure or function (10,11).

Fruits and hence fruit juices and vegetables are valuable sources of minerals. Diets high in fruits and vegetables are also linked to decreased risk of diseases (diabetes, cancer, etc.) and their consumption should be encourage (10). This study was directed to determine the concentrations of some trace elements, namely Zn, Fe, Cu, Mn, Co, Cr, and Ni in fruit juice available commercially in and around Jeddah city. A total of 129 samples of apple, orange and mango juice of 15 different brands were analyzed after 'wet digestion' for seven trace elements using Graphite Furnace Atomic Absorption Spectrometer (GFAAS). The obtained metal concentrations are compared with the corresponding values of different countries available in the literature.

## MATERIALS AND METHODS

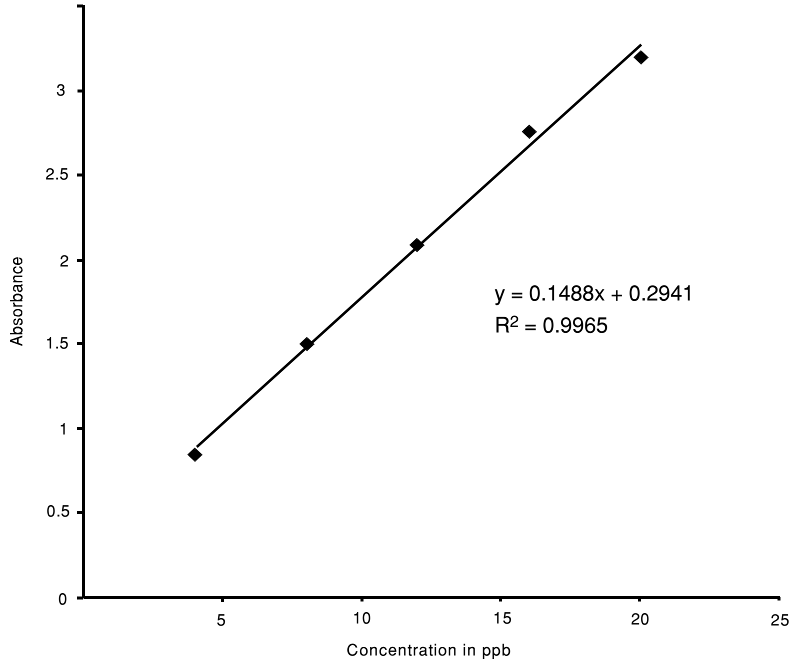
### Collection of fruit juice samples

For the present study, commercially available fruit juices samples of three different types (apple, orange and mango) of 15 different brands were bought from different supermarkets in Jeddah City ; the most frequently consumed brands were selected. A total of 129 juice samples were analyzed in the present study.

Table 5: Comparison of daily intakes of metals from 0.0274 l of fruit juice by Saudi population with the recommended values from all food intake.

| Element | Apple Juice (µg) | Orange Juice (µg) | Mango Juice (µg) | Recommended/Permissible value (mg/day) or ( g/day) |
|---|---|---|---|---|
| Zn | 14.93 | 25.25 | 14.13 | 11 mg (Male)<br>8 mg(Female) |
| Fe | 9.27 | 10.20 | 13.46 | 8 mg (Male) |
| Cu | 9.05 | 14.11 | 13.39 | 2 - 3 mg |
| Mn | 0.18 | 0.17 | 0.22 | 50 - 200 µg |
| Co | 0.23 | 0.22 | 0.24 | 40 µg |
| Cr | 0.67 | 0.59 | 0.63 | 2.5 - 5 mg |
| Ni | 0.18 | 0.16 | 0.17 | 100 -300 µg |

Figure 1: Concentration versus absorbance calibration curve for Ni.




### Reagents and glass wares

Atomic Absorption Spectroscopic Standard solutions for Zn, Fe, Cu, Mn, Co, Cr, and Ni were purchased from Fisher Scientific Company, USA. Working standard solutions were prepared by diluting the stock solution. All solutions were prepared with double - distilled deionized water obtained by filtering distilled water through a Milli - Q purifier system (Millipore, Direct-Q 5, France) immediately before use. Samples were mineralized with 65% nitric acid ($HNO_3$) (Merck Suprapure) and vanadium pentoxide ($V_2O_5$) (Merck, analytic grade). All glass wares (conical flask, volumetric flask, watch glass, pipette, measureing cylinder, *etc.*) were of borocylicate (England). All glass wares, before and after use, were washed with double - distilled water, then soaked in nitric acid solution at 30% (v/v) during 24 h, rinsed several times in double - distilled de-ionized water and dried in air. Items were kept in a clean place to avoid contamination.

### Sample digestion and preparation of analyte solution for AAS

Prior to quantitation of analyte by Atomic Absorption Spectroetry it is usually necessary to destroy the organic matrix and bring the element into clear solution. For this reason the juice sample was first digested with chemicals where the organic matrix of juice was destroyed and left the element into a clear solution. 'Wet Digestion' method (12-15) has been used in the present study. All homogenized samples (5 ml) were treated with 1 ml of $HNO_3$ and a few grams of $V_2O_5$ in a multiplace mineralized block. Digestion was completed in 90 minutes at 120°C. When cool, the digest was diluted to a 10 ml volume with double - distilled deionized water.

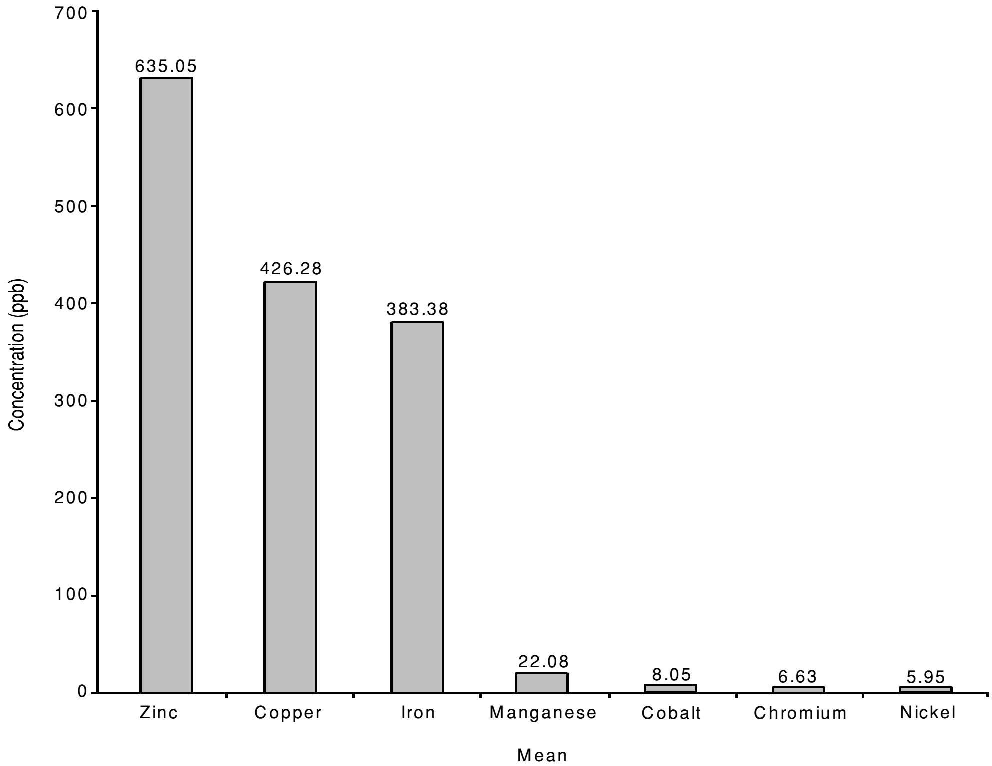
### Apparatus and Calibration

We used the VARIAN Graphite Furnance Atomic Absorption Spectrometry (GFAAS), Model Spectra AA 30P, equipped with a deuterium background corrector. The description of machine and the method to calibrate the AAS machine for determining the elemental concentration were reported in our earlier paper (16). The sensitivity of the AAS machine was also tested and reported earlier (16).

### Measurement of elemental concentration in fruit juice samples

10 µl liquid of each juice samples, obtained after wet digestion was injected into the graphite tube of the AAS with the help of an auto-sampler, and the elemental concentration was read from the output of the printer connected to the computer associated with the AAS machine. Each sample was repeated several times for each element and the average was recorded.

Figure 2: Distribution of concentration of seven trace elements in fruit juice.




The concentration of Zn, Fe, Cu, Mn, Co, Cr, and Ni were determined for each sample of fruit juice. A total of 129 samples of 15 different brands were analyzed in the present study. Out of 129 juice samples, 42 were apple juice samples, 45 orange juice samples and 42 were mango juice samples.

**Quality Control**

Appropriate quality assurance procedures and precautions were carried out to ensure reliability of the results and were reported earlier (16). Samples were generally carefully handled to avoid contamination. The recovery test of the total analytical procedure was also carried out for some of the metals in selected samples by spiking analyzed samples with aliquots of metal standards and then reanalyzing the samples (12,17). Acceptable recoveries of 94 ± 1% and 96 1% were obtained for chromium and copper respectively.

## RESULTS AND DISCUSSION

The results of our measurements of the elemental concentrations in standard reference milk sample (A-11) obtained from IAEA were reported earlier (16). The result agreed within 7% of the certified values.

The range of linearity of concentration versus absorbance graph is of great importance in determining

| | |
|---|---|
| Zinc | $y = 0.0096\,x + 0.0915$, $R^2 = 0.9981$ |
| Copper | $y = 0.009\,x + 0.1075$, $R^2 = 0.9982$ |
| Iron | $y = 0.0008\,x + 1.069$, $R^2 = 0.9895$ |
| Chromium | $y = 0.0053\,x + 0.0105$, $R^2 = 0.9944$ |
| Manganese | $y = 0.0396\,x + 0.0246$, $R^2 = 0.9995$ |
| Cobalt | $y = 0.0251\,x + 0.037$, $R^2 = 0.9931$ |
| Nickel | $y = 0.1488\,x + 0.2941$, $R^2 = 0.9965$ |

the elemental concentration of the juice samples. The calibration graphs obtained for Zn, Fe, Cu, Mn, Cr, and Co were reported earlier (16). The calibration graph for Ni is shown in Figure 1.The adjusted linear equation and correlation coefficients are:

The observed correlation coefficients were also assessed using Student's t - test at 5% level of significance. The results showed that there is significant correlation between the variables for each element under study.

The results of the present study for seven trace elements in fruit juice are given in Table 1. In apple juice, the concentration of Zn is the highest while that of Ni is the lowest. The same is also true for orange and mango juice samples.

The mean of each element in three types of fruit juice samples is shown in the last column. The mean concentration of Zn in fruit juice is highest followed by Cu, Fe, Mn, Co, Cr and the last Ni. The distribution of concentrations of seven trace elements in fruit juice is shown in Figure 2.

There are wide variations in published data for the elemental concentrations of fruit juice of different countries (9,10,12-20, 30-34). Some of the results are recorded in Table 2 for apple juice for comparison with the present values. Table 3 shows the comparison of the presenet values of orange juice with the corresponding values of different countries. Table 4 shows the comparison for mango juice. Only few results are available in literature for mango juice. In general, our values are well comparable with the published results of different authors.

The daily intake of metals depends on both the concentration and the amount of food consumed. The reported values of orange juice consumption in USA and UK are respectively 20 liter per person a year and 14 liter per person a year (21). The consumption of fruit juice in Spain is 13.21 liter per person per year (12,22,23).

Assuming a value of 10 liter per person a year for fruit juice consumption in Saudi Arabia, the daily intake of these metals are determined and depicted in Table 5. The last column shows the Recommended Dietary Allowance (RDA) / Tolerance values as set by different international organizations (24-29). It is evident that the daily intake of these metals are well below the recommended / tolerable values.

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Correspondence:
Syed M. Farid
Nuclear Engineering Department,
King Abdulaziz University,
P.O. Box 80204, Jeddah-21589,
SAUDI ARABIA.
e-mail: smfarid44@hotmail.com