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Original Article

Assessment of Heavy Metals Concentration and Microbial Profile in Sudanese Carbonated Soft Drinks and Beverages

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Abstract - *The present study was conducted in the Faculty of Agriculture at Elnelien University during the period 2020 – 2022. The objective of the study was to determine the heavy metal and microbial profile of Sudanese carbonated soft drinks and beverages, and to determine whether they coincide with WHO (2011) standards. The data of samples were statistically analyzed by using the Complete Randomized Design (CRD), and the mean separation was done by the Least Significant Difference Test (LSDT). Firstly, Heavy metals recorded in soft drinks were Pb, and it was found in Sprite, Fanta, Pepsi and Miranda, and the values were above the permissible limit of 0.01 ppm, while in all companies, no Iron (Fe), Copper (Cu), Cadmium (Cd) and Chromium (Cr) metals were reported. Heavy metals recorded in beverages were Fe and Pb, and they were found below the permissible limit of 0.3 and 0.01, respectively, except for Pb content in Rio Mango, which was found to be above the permissible limit. In all beverages, no Copper (Cu), Cadmium (Cd), and Chromium (Cr) metals were reported. The microbial profile of soft drinks, beverages and water showed that total bacteria count, total fungal count, E. coli and Salmonella recorded negative results. It is concluded that no microbial hazards in the drinks. Therefore, it can be concluded that no microbial hazards in the beverages and waters. In case of soft drinks, the only heavy metal recorded was Pb and it was found in Sprite, Fanta, Pepsi and Miranda and the values were above the permissible limit, only heavy metals recorded in beverages were Fe and Pb and they were found below the permissible limit except Pb content in Rio Mango was found to be above the permissible limit. It was found that quality parameters in most soft drink samples were within the critical limit, so the net content end product quality parameters of all beverages and water are ok. It is recommended that more elaborate work be done on the general requirements of the food safety program.*

Keywords *- Heavy metal, Microbial profile, Carbonated soft drink.*

1. Introduction

Carbonated Soft Drinks (CSDs) are enjoyed globally on a daily basis due to their sharpness, mouth feel, flavor, refreshing qualities, and ability to quench thirst. According to Sudanese standards [1, 2], CSDs must be free from E. coli bacteria, pathogenic bacteria, as well as yeast and mold cells. Furthermore, the Sudanese regulations stipulate that CSDs should be bottled in hygienic conditions, which encompass the buildings, equipment, packaging materials, and machinery utilized in the process. Additionally, the presence of certain heavy metals such as cadmium, lead, mercury, arsenic, and zinc in soft drinks has been documented, potentially resulting from environmental pollution of surface and underground water and the food and fruits used during production [3]. Heavy metals are recognized as harmful and toxic to the human body [4], posing a significant public health risk [5]. These metals can induce both acute and chronic toxicity through various mechanisms in individuals of all ages, including children and adults [6, 7].

Some heavy metals serve as catalysts in oxidative reactions involving biological macromolecules, and their intoxication can result in oxidative damage to tissues [8]. Cadmium is a heavy metal that, upon long-term accumulation, may lead to cancer, as it is classified as a carcinogenic element [8]. Moreover, prolonged exposure to cadmium can result in its accumulation in the kidneys and liver due to its extended biological half-life, potentially causing kidney damage [9]. Lead is known to impact humans and animals across all age groups, but its effects are particularly severe in young children [10]. The

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most prevalent manifestation of lead poisoning in children is central neurotoxicity [11]. Other symptoms associated with childhood lead toxicity include anemia, peripheral motor neuropathy, and gastrointestinal issues such as anorexia, vomiting, and abdominal pain, as well as growth delays [12].

2. Materials and Methods

The research conducted encompassed all Sudanese carbonated soft drink brands, specifically the Dal group, Arak group, Elniel, Elberer group, Fowaz, and Pasgianos companies, which graciously provided all samples in Khartoum, Sudan. All other chemicals and reagents were used as received, adhering to analytical reagent grade standards. The carbonated soft drink samples were collected in triplicate from the factories during the observational audit. The samples were stored in dry bottles, securely sealed and appropriately labeled. Subsequently, all samples were transported to the analytical laboratory to fulfil the requirements for food safety and food quality assessment.

2.1. Heavy Metal Concentration of Carbonated Soft Drinks and Beverages

Concentration of heavy metals in carbonated soft drinks and beverages: Heavy metals present in soft drinks, beverages, and water were measured using an atomic absorption spectrometer, following the procedure outlined by AOAC [14]. Afterwards, 10 ml of nitric acid (69%) was combined with 10 ml of the sample, and the resulting mixture was evaporated on a hot plate within a fume cupboard until the brown fumes dissipated, leaving only white fumes. Distilled water was added to adjust the volume to 25 ml, which was filtered and prepared for analysis using AAS.

2.1.1. Preparation of the Standards Curve

Merck (Darmstadt, Germany) provided standard solutions for the heavy metals being analyzed, which include lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), and nickel (Ni). The individual 1000 mg kg−1 standards (Merck) were utilized to formulate the standards in 0.1 N HNO3. Calibration curves for the metals were established by selecting 0–40 µ/L as necessary for the calculations, and working standards were generated from the previous stock solutions by diluting them with 0.1 N HNO3 until the desired concentrations for analysis were achieved.

2.1.2. Analysis of Heavy Metals in Carbonated Soft Drinks and Beverages

All measurements for metals (Fe, Pb, Cu, Cd, and Cr) were conducted using an air/acetylene flame (AA). The wavelengths of the primary resonance lines were 243.3, 283.3, 324.228.8, and 357.9 nm, respectively. In Atomic Absorption Spectroscopy (AAS), absorbance is directly proportional to concentration. Consequently, to determine the concentration of metals in samples of soft drinks and beverages via AAS, the sample volume, ramp, and hold time for digestion were optimized prior to analysis to achieve maximum absorbance and minimal background interference. The application of a HNO3/HCl mixture during the digestion of samples facilitates the determination of the total heavy metal content analyzed in all samples. Beginning with the least concentrated solution, the standard metal solutions were successively aspirated into the flame, followed by the test solution, with absorbance recorded in each instance.

2.2. Microbiological Analysis of Carbonated Soft Drinks and Beverages

Microbiological examination of carbonated soft drinks and beverages: Microbiological analysis was conducted using the membrane filtration technique, which adheres to the standard procedure established by the AOAC [14].

As Detailed Below

All equipment underwent disinfection; forceps, pipette, and glassware were sterilized using an autoclave. All surfaces were cleaned with a 70% ethanol solution, and ultraviolet light was utilized in the clean chamber device. A flame was maintained throughout the experiment. Protective gloves, clothing, and a hairnet were also worn. PET bottles were thoroughly washed and wiped down with a 70% ethanol solution before examination.

2.2.1. Total Plate Count of Carbonated Soft Drinks and Beverages

The plate count was assessed utilizing the membrane filtration technique in accordance with the AOAC standard method [14]. A pack of ten was opened, and a Petri dish containing a nutrient pad medium was taken out. To the Plate count-NPS-nutrient pad in the Petri dish, 3 – 3.5 ml of sterile, distilled, or dematerialized water was added. The moisture level is considered optimal if a distinct ring of liquid is visibly present. An envelope was unsealed, and a 0.45 µm green, gridded membrane filter (20/41 ST) was extracted using sterile tweezers. This membrane filter was positioned atop the filter holder, and the filter funnel was subsequently placed on top. The sample was filtered by activating the pump. Following this, the membrane filter was aseptically and cautiously detached from the frit using sterile tweezers and placed onto the prepared nutrient pad, ensuring no air bubbles were trapped. The Petri dish was incubated with the lid facing upwards for 48 hours ± 3 at an incubation temperature of 35 °C ± 1°C.

2.2.2. Total Yeasts and Molds in Carbonated Soft Drinks and Beverages

Yeasts and molds were assessed using the membrane filtration technique in accordance with the AOAC standard method [14]. A pack of ten was opened, and a Petri dish containing a nutrient pad was taken out. Medium 3 – 3.5 ml of sterile, distilled, or demineralized water was added to the selective medium - "Malt Extract-NPS," designed to identify yeast and mold in beverages - nutrient pad within the Petri dish. The moisture level is considered optimal if a distinct ring of liquid is visible.

The sealed envelope was opened, and a 0.6 µm black, gridded membrane filter 26/31 ST was carefully extracted using sterile tweezers. This membrane filter was then positioned atop the filter holder, and the filter funnel was placed on top. The sample was filtered by activating the pump. Subsequently, the membrane filter was aseptically and gently removed from the frit using sterile tweezers and placed onto the prepared nutrient pad, ensuring no air bubbles were trapped. The Petri dish was incubated with the lid facing upwards for 120 hours ± 3 incubation periods at a temperature of 25 °C ± 1°C.

2.2.3. Total Coliform and E. coli Levels in Carbonated Soft Drinks and Beverages

Total coliform bacteria were assessed using the Membrane filtration method as per the AOAC standard method [14]. A pack of ten was opened, and a Petri dish containing a nutrient pad was taken out – 3 – 3.5 ml of sterile, distilled, or dematerialized water was added to the selective medium "Coli chrome -NPS," which is designed for the rapid quantitative detection of E. coli and coliform bacteria through optical differentiation within 24 hours on the nutrient pad in the Petri dish. The moisture level is considered optimal if a distinct ring of liquid is visible.

A sealed envelope was opened, and a 0.45 µm white, gridded membrane filter ME 25/21 ST was carefully extracted using sterile tweezers. This membrane filter was positioned atop the filter holder, and the filter funnel was subsequently placed on it. The sample was filtered by activating the pump.

The membrane filter was then aseptically and gently removed from the frit using sterile tweezers and placed onto the prepared nutrient pad, ensuring no air bubbles were trapped. The Petri dish was then incubated with the lid facing upwards for a duration of 24 hours ± 3 incubation periods at a temperature of 35 °C ± 1°C. Note: E. coli is indicated by blue colonies, while coliform bacteria are represented by red colonies.

2.2.4. Salmonella Bacteria in Carbonated Soft Drinks and Beverages

Nutrient broth was utilized for the selective isolation and detection of Salmonella bacteria. The setup was sterilized through autoclaving at 121 °C for 15 minutes. Three consecutive decimal dilutions of each sample were created.

For each decimal dilution, three tubes of Nutrient broth were inoculated with 1 ml of the corresponding solution and subsequently incubated at 37 °C for 48 hours. The contents of each positive tube were then cultured in a new silent cystine broth and incubated at the same temperature (37 ± 0.25 °C) for 24 hours, which constitutes the Eijkman test.

The production of gas and acid indicates the presence of Salmonella bacteria in the sample. Finally, to confirm the presence of Salmonella, each positive tube in BSA was cultured on Bismuth Sulphite Agar in three Petri dishes corresponding to each positive tube and then incubated at the same temperature (37 ± 0.25 °C) for 24 hours. This is referred to as the complete test if the colonies appear dark gray, indicating the presence of Salmonella.

2.3. Statistical Analysis

The obtained data were stored in Microsoft Excel 2007 and then exported into SPSS Version 19.0 software (SPSS Inc., USA) for statistical analysis. The data of samples were statistically analyzed by using the Complete Randomized Design (CRD), and the mean separation was done by the Least Significant Difference Test (LSDT).

3. Result and Discussion

3.1. Heavy Metals in Sudanese Soft Drink Samples

Table1. demonstrated some heavy metal content in soft drink samples collected from Sudanese soft drink industries as compared with the maximum permissible limit.

In case of Coca - Cola company soft drinks, Sprite and Fanta recorded 0.066 ppm and 0.016 ppm respectively lead (Pb) content these values were above the permissible limit (0.01 ppm), in case of Pepsi company soft drinks, Pepsi recorded 0.061 ppm of lead (Pb) and Miranda recorded 0.034 ppm of Pb and each value was above the permissible limit (0.01 ppm) while soft drinks of Stim and Pasgianos companies recorded no Pb content.

In all companies, no Iron (Fe), Copper (Cu), Cadmium (Cd) or Chromium (Cr) metals were reported. Therefore, it can be concluded that the only heavy metal recorded was Pb, and it was found in Sprite, Fanta, Pepsi and Miranda, and the values were above the permissible limit.

3.2. Heavy Metals in Sudanese Beverage Samples

Table2. demonstrated some heavy metal content in beverages and water samples collected from Sudanese soft drink industries as compared with the maximum permissible limit. In case of Coca – cola company beverages, Rio-Guava recorded 0.109 ppm iron (Fe) content which was below the permissible limit (0.3ppm) and 0.001 ppm lead (Pb) that was below the permissible limit (0.01ppm), Rio-Mango recorded 0.137 ppm iron (Fe) content which was below the permissible limit (0.3ppm) and 0.013 ppm lead (Pb) that was above the permissible limit (0.01ppm) In case of Pepsi company beverages, Crystal Guava recorded 0.286 ppm iron (Fe) content which was below the permissible limit (0.3ppm), refer to Stim company, Yes Guava recorded 0.110 ppm iron (Fe) content which was below the permissible limit (0.3ppm).

In the case of Fouz company beverages, Vita Guava recorded 0.108 ppm iron (Fe) content which was below the permissible limit (0.3ppm) and 0.006 ppm lead (Pb) that was below the permissible limit (0.01 ppm), Vita Mango recorded 0.10 ppm iron (Fe) content which was below the permissible limit (0.3ppm). In all companies, no Copper (Cu), Cadmium (Cd) or Chromium (Cr) metals were reported. Therefore, it can be concluded that the only

heavy metals recorded were Fe and Pb, and they were found below the permissible limit, except Pb content in Rio Mango, which was found to be above the permissible limit.

3.3. The Microbiological Profile of Sudanese Soft Drinks and Beverage Samples

The microbial profile was examined for all samples (soft drinks, beverages and water) collected from Sudanese soft drink industries. The total bacteria count CFU / ml Coca–Cola company soft drinks, which include Coca, Sprite and Vita, Pepsi soft drinks, which include Pepsi, 7Up and Miranda, Stim soft drinks, which include Stim, Royal and Champion, Pasgianos soft drinks, which include Pasgianos and Assel recorded –ve results. The total fungal count of CFU / ml, E. coli, and Salmonella was also recorded as negative in all soft drinks produced by the companies. Therefore, it can be concluded that all samples were free from microbial hazards.

Table 1. Determination of some Heavy metal content in soft drink samples collected from Sudanese soft drink industries, compared with the maximum permissible limit of the WHO (2011)

Heavy metals	limit range (ppm)	Coca-Cola			Arak			Stim			Pasgianos	
		Coca	Sprite	Fanta	Pepsi	7-Up	Miranda	Stim	Royal	Champion	Pasgianos	Assel
Fe	0.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pb	0.01	ND	0.066	0.016	0.061	ND	0.034	ND	ND	ND	ND	ND
Cu	2.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cd	0.003	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cr	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 2. Determination of some Heavy metal content in beverage samples collected from Sudanese soft drink industries compared with the maximum permissible limit of the WHO (2011)

Heavy metals	limit range (ppm)	Sudanese soft drink industries								
		Coca-Cola		Pepsi		Stim		Pasgianos	Fouz	
		Rio Goafa	Rio Mango	Mango Goafa	Crystal Mango	Royal	Yes Goafa	Frat orange	Vita Goafa	Vita Mango
Fe	0.3	0.109	0.137	0.286	ND	ND	0.110	ND	0.108	0.100
Pb	0.01	0.001	0.013	ND	ND	ND	ND	ND	0.006	ND
Cu	2.00	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cd	0.003	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cr	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND

4. Conclusion and Recommendations

4.1. Conclusion

- It is concluded that the only heavy metal recorded was Pb, and it was found in Sprite, Fanta, Pepsi and Miranda, and the values were above the permissible limit.
- Only heavy metals recorded in the beverage were Fe and Pb, and they were found below the permissible limit, except Pb content in Rio Mango was found to be above the permissible limit.
- The microbial profile of soft drink samples collected from Sudanese soft drink industries, total bacteria count, Total fungal count, *E. coli* and Salmonella recorded negative results (–ve) in all soft drinks produced by the companies. Therefore, it can be concluded that no microbial hazards in the drinks.
- The microbial profile of beverage samples collected from Sudanese soft drink industries, total bacteria count, Total fungal count, E. coli and Salmonella recorded negative results (–ve) in all beverages produced by the companies. Therefore, it can be concluded that no microbial hazards in the beverages.

4.2. Recommendations

- Extra research on quality parameters for soft drinks and beverages.
- More studies are recommended to minimize the concentration level for heavy metals such as Pb and Fe to comply with the recommended range.

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