Assessment of Heavy Metal Contamination (Nickel and Arsenic) using GF-AAS in Local Brand Chocolates and Candies from Tiruchirappalli, India

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ABSTRACT
Nineteen different varieties of local brand chocolates, soft candies and hard candies were procured from Tiruchirappalli district, Tamil Nadu. These samples were analyzed for two heavy metals nickel and arsenic in Graphite Furnace – Atomic Absorption Spectrophotometer (GF-AAS). Out of 19 local brands of chocolates and candies, five were chocolate type, six were soft candy type and eight were hard candy type. From the analysis, it was found that nickel levels in the samples ranged from 0.77 to 5.47 µg/g with an average of 2.75µg/g and arsenic levels ranged from 0.01 to 4.50 µg/g with an average of 0.81 µg/g. Concentration of nickel was high in chocolates and hard candies. Local brand soft candies revealed the highest concentration of arsenic. Results showed that there is significant heavy metal contamination in the collected confectionary samples and on frequent consumption it will cause serious health effects in children and adults as well.

General Terms
Local brand chocolates, Heavy metals, Toxicity.

Keywords
Chocolates, Candies, Nickel, Arsenic, GF-AAS.

1. INTRODUCTION
Heavy metals enter into human body by ingestion, inhalation and absorption through skin or mucous membrane, when they are not metabolized by the body, gets accumulated in soft tissues and become toxic. Due to the industrial revelation, heavy metal is easily affects the human. The presence of relatively high concentrations of heavy metals in a consumer product that is marketed to children is of an extraordinary concern [1] because children are the most sensitive and vulnerable age group to any kind of contamination in the food chain [2].

While chocolate is regularly eaten for gratification, there are potential beneficial health effects of eating chocolate; the unconstrained consumption of large quantities of any energy-rich food such as chocolate without a corresponding increase in activity could increase the risk of obesity and dental complications. Cocoa solids contain alkaloids such as theobromine and phenethylamine, which have strong physiological effects on the body. These alkaloids have been linked to serotonin levels in the brain. Some research found that chocolate, consumed in controlled quantity, can lower blood pressure. Candy has a high Glycemic Index (GI), which means that it causes a rapid rise in blood sugar levels after ingestion. This is of major concern for people with diabetes, but could also be risky to the health of non-diabetics. Many different studies and techniques for heavy metal determination in different food stuffs have been reported. Mainly, dry, wet and microwave digestion methods are used to investigate heavy metal levels in cheese, fruits, fruit juices, vegetables, sweets, chocolates, honey, snacks, appetizers, etc. [3-7]. The second French TDS (Total Diet Study) estimated dietary exposure to 28 essential and non-essential elements in 1319 different food samples [8].

Concentrations of cadmium, copper, lead and arsenic were investigated in cocoa beans from various countries as well as in some chocolate products, where high concentration of cadmium content was reported [9, 10]. Arsenic plays an essential role in human because decrease or increase in serum arsenic concentration has been correlated with injuries of the central nervous system, vascular disease and cancer [11]. Nickel content in Indian foods is much higher (240–3,900 µg /day) [12]. There is no information about maximum nickel levels in chewing gums and candies in Turkish standards; however there are reports on high levels of nickel in some food samples [13]. Cocoa butter is one more important ingredient contains high concentrations of nickel [14]. So in this study the main focus was to analyze the heavy metal concentrations (nickel and arsenic) in local brand chocolates and candies using GF-AAS.

2. MATERIALS AND METHODS
2.1 Sample Collection
The collected 19 samples were purchased from suburban areas of Tiruchirappalli district, Tamil Nadu, India. They were divided into three types such as five varieties of chocolates, six varieties of hard candies and eight varieties of soft candies. Samples were stored in polypropylene plastic bags until further analysis.

2.2 Reagents
All reagents were of analytical reagent grade unless otherwise stated. Before going for digestion of samples, the glasswares and AAS polystyrene disposable sample cups were soaked

overnight and cleaned thoroughly in freshly prepared 1% HNO_3 solution. Working standards for Ni and As were prepared by diluting concentrated stock solutions (Merck, Darmstadt, Germany) of 1000mg/L with 0.5 M HNO_3.

2.3 Sample Preparation for Wet Digestion

Wet digestion was carried out, where one gram each of chocolate, soft candy and hard candy samples were weighed and placed in a 50ml Erlenmeyer conical flask containing concentrated HNO_3 and H_2O_2. This mixture was heated for 10 min at 100°C in microwave oven until the solubilisation of the samples were completed. After cooling the Erlenmeyer flask, the resulting solutions were evaporated to semidried mass to remove the excess acid, and then diluted to 10ml in volumetric flask with distilled water.

2.4 Sample Analysis

All digested samples were analyzed in triplicate using a GF Atomic Absorption Spectrophotometer (Thermo Scientific ICE 3000 series) to determine Ni and As concentrations. This system was operated using the wizard driven Thermo scientific SOLAAR data station V 11.02 software. Blanks and calibration standard solutions were also analyzed in same way as the samples. For graphite furnace measurements, argon was used as inert gas. The instrument's operating conditions are shown in Table 1.

Table 1. GF-ASS parameters for analyzing nickel and arsenic

S. No	Parameters	Nickel	Arsenic
1.	Lamp Serial number	9614003	9618003
2.	Lamp current (mA)	15	12
3.	Wave length (nm)	232.0	193.7
4.	Instrument mode	Furnace	Furnace
5.	Gas flow(µg/l)	0.2	0.2
6.	Band width (nm)	0.2	0.5
7.	Furnace programme total time in (sec)	72.0	73.3

3. RESULTS AND DISCUSSION

The nickel concentration ranged from 0.77 to 5.29 µg/g with an average of 2.9 µg/g in the 5 varieties of chocolate (Figure 1). Concentration ranged from 1.02 to 4.23 µg/g with an average of 1.62 µg/g in 6 varieties of soft candies and concentration ranged from 1.08 to 5.47 µg/g with an average of 3.7µg/g in 8 varieties of hard candies. Nickel is the main known contaminant resulting from the manufacturing process of chocolate, when hardening is done by hydrogenation of unsaturated fats using nickel as catalyst [2]. Candies, chocolates and hard candy showed elevated levels of nickel than the normal value. Sources of metal contamination were mainly from the raw materials used in manufacturing processes and leaching of metals from the vessel in which they are stored. Nickel is considered to be a normal constituent of diet and its compounds are generally recognized as safe when used as a direct ingredient in human food [15]. Little is known about the actual chemical forms of nickel in various foods or whether dietary nickel has distinct 'organic' forms with enhanced bioavailability analogous to those of iron and chromium. Nickel levels in food stuffs generally range from less than 0.1mg/kg to 0.5mg/kg [16]. In suburbs of Mumbai, presence of heavy metals was determined in different brands of chocolates and candies. In this study, nickel was in the range of 0.041 – 8.29 µg/g with an average of 1.63 µg/g. As per the International Atomic Energy Agency (IAEA), the certified value of nickel in a reference material is 4.0 (3.8 – 4.9 µg/g) [2]. According to literature, few foods (Dray beans, Eggs, Wheat, Baking soda and fruits) have been contaminated with nickel during the manufacturing process but mostly occur naturally [17].

Rich food sources of the nickel include oat, meal, dried beans, peanuts, dark chocolates and soya products and consumption of these products in larger amounts may increase the nickel intake to 900 µg/person/day or more [18]. Human nickel exposure originates from a variety of sources and is highly variable. Nickel is normally present in human tissues and under conditions of high exposure these levels may increase significantly [19, 20]. Food processing methods apparently added the nickel level already present in foodstuffs *via*, 1. Leaching from nickel containing alloys in food processing equipment made from stainless steel; 2. Milling of flour; 3. Catalytic hydrogenation of fats and oils by use of nickel catalysts [16, 21]. Food intake, gastric emptying and peristalsis of the intestine are of substantial significance for the bioavailability of nickel, because absorption of ingested nickel is lower than the level when it is administrated in food or in water together with a meal. The presence of food in the stomach significantly alters the bioavailability of nickel salts [11, 22, 23].

In the present study, the arsenic concentration ranged from 0.03 to 0.90 µg/g with an average of 0.41 µg/g in 5 varieties of chocolate (Figure 2). Concentration ranged from 0.10 to 4.50 µg/g with an average of 1.18 µg/g in 6 varieties of soft candies and concentration ranged from 0.01 to 2.88 µg/g with an average of 0.73 µg/g in 8 varieties of hard candies. Arsenic is a metal that occurs at ultra trace levels, for which its specific biochemical function has not totally been well defined. Circumstantial evidence suggests that dietary deprivation results in a suboptimal biological function that is prevented or reversed by an intake of physiological amounts of the element. It has been suggested that this metal could play an essential role in humans because decrease in serum arsenic concentration has been correlated with injuries of the central nervous system, vascular disease and cancer [11]. The concentration of arsenic studied in the chocolates and candies samples were 10% greater than the normal value that is given by Turkish standard [24]. According to a study, the mean concentration of arsenic in bakery goods in arsenic affected areas in West Bengal was around 130-170 µg/kg. The provisional tolerable daily intake As value for adult is 11.8 – 13.9 µg/kg body wt/day (FAO/WHO report – 2.1 µg/kg body wt/day) [25]. It is necessary to study the consumption habits of children, because of the adverse effects of the raw materials, utensils which were used during production of the confectionary products. It is known that even the prolonged intake of low concentrations of arsenic can causes skin, lung, kidney and bladder cancers [26]. Consequently, the concern in analyzing total arsenic levels in foodstuffs has increased [27].

4. CONCLUSION

Many kinds of chocolate and candy products which are consumed frequently by small children and most of them are sold at retail stores near elementary schools and rural areas. Generally, wrappers with colourfully printed outer covers are used as packages of candy products in order to attract small children to purchase them. Most of the pigments of the printing inks are based on metallic compounds such as Zn, As, Ni, Ag, Cu, Pb, and Cr. However, harmful metals such as As, Pb, Ni and Cr have been prohibited by law in most of the countries from being used in food packaging. In this study, heavy metal determination using GF-AAS was performed. Our results showed the presence of heavy metals in considerable concentration in the local brand candies and chocolates. It could be concluded that daily or frequent consumption of the local brand chocolate and candies could indirectly aid in heavy metal intake which is harmful for the children. In general, restricting children to eat chocolates and candies could help in maintaining oral health and in particular, avoiding local brand chocolates and candies, could prevent consumption of heavy metals indirectly.

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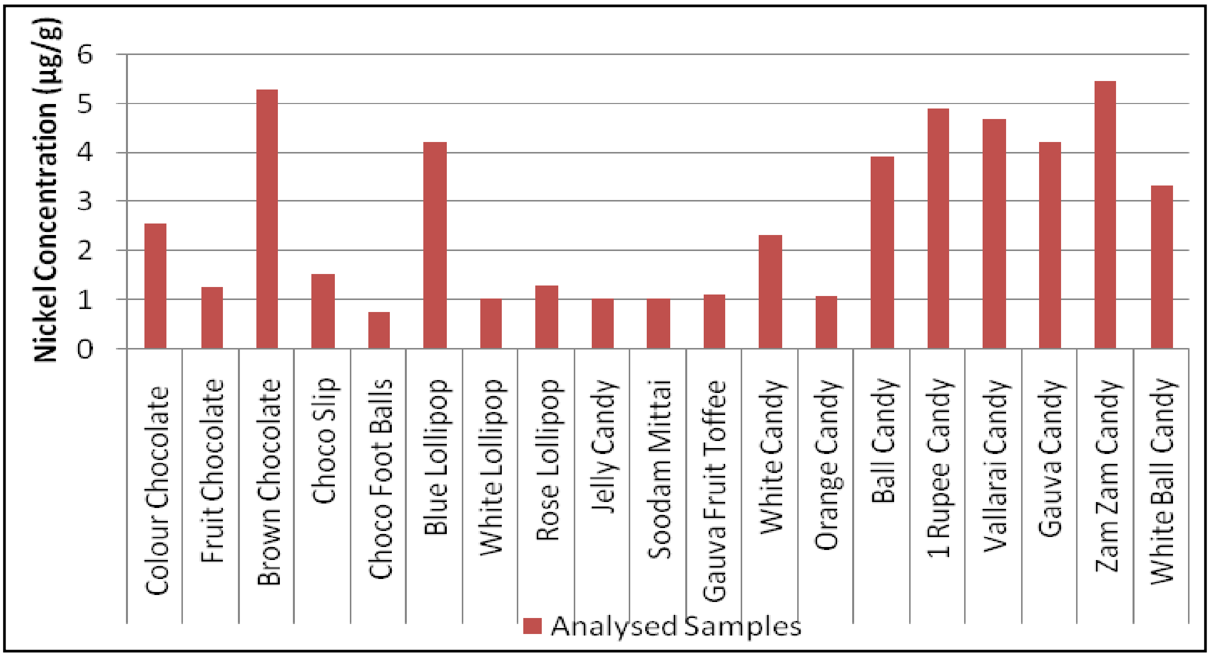

Fig 1: Graphical illustration of nickel present in 19 samples using GF-AAS

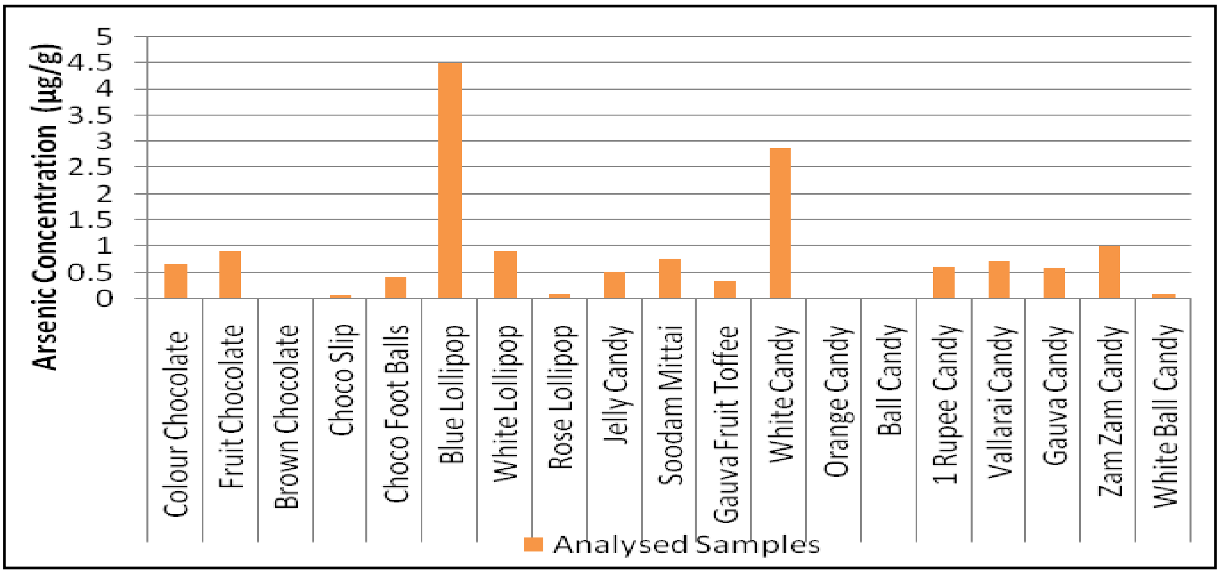

Fig 2: Graphical illustration of arsenic present in 19 samples using GF-AAS

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