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Determination of Toxic Metals in Chocolate Confectionery Wrappers Used by the Chocolate Manufacturers in Sri Lanka, and It's Migration to Chocolates Under Different Storage Conditions

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Abstract: ***Chocolate is one of the most popular confectioneries consumed by all age groups. Among them children are the most attracted group of consuming chocolates and, at the same time the most vulnerable for toxic metals. Toxic metals can accumulate in the body even consumption of small amount of metals, leading to neurotoxin, carcinogenic, and brain disorders. Due to their frequent hand-to-mouth behavior, children can be easily ingested by toxic metals. Considering this major risk, it is significant to asses the toxic metals that could be present in chocolates and its wrappers. 48 samples were analyzed to determine the total Chromium, Nickel, Arsenic, Cadmium, Antimony and Lead. Samples were stored under room temperature and refrigerated conditions and acid digested and analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Total Chromium, Nickel, Antimony and Lead were detected in higher concentrations in chocolate confectionery wrappers, and found in low concentrations in chocolates. Arsenic and Cadmium were not detected neither in chocolates nor wrappers. There were no significant difference between the concentrations in room temperature stored chocolates (30-31^{0}C), and refrigerated chocolates (10-12^{0}C), but the migration of toxic metals are more favorable in room temperature.***

Keywords: Toxic Metals, Confectionery Wrappers, ICP-MS, Migration, Chocolate

1. Introduction

"Chocolate" is a sweet, confectionery of *Theobroma cacao* seeds. The flavour of chocolate differs depending on the ingredients used and the preparation method of chocolate. Since chocolates are more popular among children, manufacturers are very concern about their competitors in the market. Due to this reason the chocolates and other confectioneries are sold in a very attractive manner, wrapped in colourful packaging materials. These packaging materials may contain non-food grade substances, printing inks which can contain toxic substances. The major danger is with the colourful pigments used in packaging materials. $PbCrO_4$ is an inorganic pigment used in paints and inks. However, most of the countries have prohibited the use of $PbCrO_4$ in food packages [11]. These toxic substances could have a potency to migrate into the food. Due to the frequent hand-to-mouth behavior, children can be easily ingested by toxic metals. Regular consumption of contaminated chocolates results in accumulation of toxic metals in human organs and may results in serious health problems. Therefore the aim of this study was to estimate the levels of toxic metals present in the chocolate confectionery wrappers and the degree of migration of these metals during the storage conditions.

2. Literature Review

2.1 Heavy Metal Toxicity

Toxicity of any metal is governed by several factors. There are interaction with essential metal, formation of metal protein complexes, chemical form of the metal element, immune status of the host and age and stage of development of the host. Its electrochemical character and oxidation state, its absorption and transport in body tissues, the stability and solubility of its compounds in body fluids, its case of excretion and reaction with functioning tissues and organelles and with essential metabolites[1]. Interaction of toxic metals and essential metals occur when metabolism of toxic metals is similar to that of the essential metal. Lead and Cadmium interact with Calcium in nervous system and skeletal system respectively. Lead replaces the zinc on heme enzymes. Metal protein complexes are formed due to detoxification of toxic metals in the body i.e. Metallothionin form complexes with Cd, Zn, and Cu [12]. Metal can interact with protein leading to an allosteric effect, or with DNA or RNA to stop normal metabolism or with unknown compounds leading to a change in physiologic process [1]. Contamination of food products with heavy metals may cause a serious risk for human health because of the consumption of even a small amount of metals can lead to considerable concentrations in human body leading to biotoxic effects. The biotoxic effects of heavy metals refer to the harmful effects of heavy metals to the body when consumed above the bio-recommended limits. The nature of effects could be acute, chronic or sub-chronic, neurotoxin, carcinogenic, mutagenic or teratogenic [10].

2.1.1 Lead and Chromium Toxicity

Lead absorbed from the food and the atmosphere is retained in tissues like lungs, liver, kidney, and bones. The short term and long term exposure to high level of lead can cause brain damage, paralysis, abdominal pain, anemia, renal disease, memory loss, damages to kidneys, reproductive and immune systems [18].

Chromium can exist as Cr (III) or Cr (VI). Cr (VI) form is highly toxic.Cr (VI) exposure has been known to be associated with cancer induction in humans, especially bronchial carcinoma and lung cancer [14], [11]. The mechanism(s) of Cr (VI)-induced carcinogenicity is within the cell may result from damage to cellular components during the hexavalent to trivalent chromium reduction process, by generation of free radicals, including DNA damage [3]. The water-insoluble Cr (VI) compounds of particulate forms are more potent carcinogens than the water-soluble ones [8], [11].

2.1.2 Antimony and Nickel Toxicity

Antimony and its compounds are widely use in many industries. The average intake of antimony from food and water was estimated to be roughly 5μg/ day [9]. Antimony toxicity can be categorized as inhalation exposure, respiratory effects, cardiovascular effects, gastrointestinal effects and dermal effects [17]. Nickel, is also a well known carcinogen to humans, by altering the DNA functions. Even though their DNA-damaging potentials are rather weak, they interfere with the nucleotide and base excision repair at low, nontoxic concentrations. For example, both water-soluble Ni (II) and particulate black NiO greatly reduced the repair of DNA. Ni (II) disturbed the very first step of nucleotide excision repair [7].

2.2 Toxic heavy metals in chocolate wrappers and their migration

Safe food packaging is very important. The EU Framework Directive 89/109/EEC states that “food contact materials shall be safe and must not transfer constituents in quantities that could endanger human health or induce an unacceptable change in the foodstuffs composition”. In the production process of thermoplastic polymers, such as polyethylene (PE),polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephtalate (PET), polycarbonate (PC), acrylonitrile-butadiene-styrene (ABS) and polyamide (PA),different catalysts may be used which can contain low levels of heavy metals. The sources for Cd and Pb are impurities originating from inorganic pigments and stabilizers. Antioxidants can contain Ni, while thermal stabilizers can contain Ni, Pb and Sb [13]. Today industries are using different colourful packaging materials to make their products attractive to the consumers without considering about the risk of food contamination. Especially food products such as candies that are likely to be consumed frequently by small children are wrapped in colorful packages in order to induce them to purchase the products. Candies and its packages that hold heavy metals, such as lead, chromium, and copper, can be put onto the children’s hands that pose danger [10], [11], due to their frequent hand-to-mouth behavior, and children are likely to be posed by ingesting these heavy metals**.** There is a general understanding that the package surface in contact with the food should be free of printing ink, that is, the package itself should form an effective barrier between the printed surface and the food, and the unintentional transfer of components of printing inks from the outer printed surface onto the food contact surfaces should be avoided. Use of printing inks only on the outer surface of the package does not ensure that it will not contaminate the food, although the inner surface of the package is coated with films such as polyethylene or polypropylene [11].

3. Materials and Methodology

3.1 Sample Selection

Two sets of chocolates from several local manufacturers were purchased from the market. Three chocolates per product were chosen randomly and those were considered as replicates. The selected chocolates were mainly bars, slabs, and assortments with attractive and colourful packaging materials. Freshly manufactured chocolates were obtained directly from the manufacturers without wrappers and it was considered as the control sample. One set of chocolates bought from the market were stored in room temperature (30-31^{0}C) and the remaining set of chocolates refrigerated (10-12^{0}C) for two months before the analysis.

3.2 Sample preparation by acid digestion

Reference methods - EPA 3052 For Microwave Digestion, and EPA 6020A For ICP-MS analysis.

3.2.1 Digestion of chocolates

Approximately 0.25 g of the homogenized sample was weighed into the microwave reaction vessel. 5 ml of 69% nitric acid was added to each vessel and allowed to complete pre reactions for approximately 5 minutes prior to sealing the vessels. Vessels were sealed and placed in the microwave digestion system (START D – MILSTONE). The samples were allowed to digest at 180^{0}C for 15 min. The digested solution was diluted with deionized water to 25ml, filtered through ash less filter papers and analyzed by ICP-MS (Agilent Technologies- 7700 series).

3.2.2 Digestion of wrappers

Chocolate wrappers were cut into small pieces and weighed approximately 0.15 g of the sample into the microwave reaction vessel. 5 ml of 69% nitric acid was added to each vessel and allowed to complete pre reactions for approximately 10 minutes prior to sealing the vessels. Vessels were sealed and placed in the microwave digestion system. The samples were allowed to digest at 180^{0}C for 30 min. The digested solution was diluted with deionized water to 25ml, filtered through ash less filter papers and analyzed by ICP-MS.

3.2.3 ICP-MS Analysis

A working standard series of 0.1ppb - 100ppb was prepared daily by 1 ppm mix element standard solution of Chromium, Nickel, Arsenic, Cadmium, Antimony and Lead. An Internal mix standard solution of Thallium and Yttrium were used with a concentration of 40ppb. Dilution solvent was 5% nitric acid. The selected analytical masses and the internal standards are summarized in Table 1.

Table 1: Masses and Internal Standard for selected metals for ICP-MS analysis

Element	*Mass*	*Internal Standard*	*Mass*
Cr	53	Y	89
Ni	60	Y	89
As	75	Y	89
Cd	111	Y	89
Sb	121	Y	89
Pb	208	Tl	205

3.3 Calculation of final metal concentration in chocolates and wrappers.

$$C_A = \frac{[a - b] \times V}{W \times 1000} \quad (1)$$

C_A – Final analyte concentration (in ppm)
a - Instrument reading for the sample
b - Instrument reading for the blank
V – Final volume of the sample (25ml)
W- Weight of the sample

3.4 Statistical Analysis

The results of the chocolate wrappers were statistically analyzed by Non-Parametrically and the significant levels were obtained by Kruskal-Wallis Test ($p<0.05$). The results of the chocolate samples were statistically analyzed by Non-Parametrically and the significant levels were obtained by Mann-Whitney Test ($p<0.05$).The Minitab version 14.0 was used for the statistical data analysis.

4. Results and Discussion

4.1Validation of the method

The procedure for the validation of the method for the determination of the toxic metals in chocolate confectionery and their wrappers comprised by determining the instrument detection limit (IDL), limit of quantification(LOQ), Instrument linearity, recovery rate and instrument repeatability. A Summary of the method validation and the method accuracy of chocolates (CH) and wrappers (WR) are summarized in Table 2 and Table 3 respectively.

Table 2: Summery of method validation of chocolates and wrappers

Analyte	*Linearity (r^2)*	*Inst. Repeatability (RSD <5%)*	*(IDL)*	*(LOQ)CH*	*(LOQ)WR*
Cr	1	2.96	0.11ppb	0.40ppm	0.62ppm
Ni	0.9996	2.59	0.08ppb	0.37ppm	1ppm
As	1	2.52	0.12ppb	0.18ppm	0.87ppm
Cd	0.9999	2.59	0.10ppb	0.30ppm	0.50ppm
Sb	0.9979	2.8	0.08ppb	0.32ppm	0.60ppm
Pb	1	2.9	0.09ppb	0.15ppm	0.50ppm

Table 3: Summery of method accuracy

Analyte	*Ref. conc*	*Accuracy- CH*		*Accuracy-WR*	
		Result	*% Recovery*	*Result*	*% Recovery*
Cr	20 ppb	18.7	93.5	18.88	94.3
Ni	20 ppb	18.23	91.15	18.6	93
As	20 ppb	18.73	93.65	18.85	94.25
Cd	20 ppb	18.76	93.8	18.18	90.9
Sb	20 ppb	18.59	92.95	18.6	93
Pb	20 ppb	18.68	93.4	18.71	93.5

4.2 Levels of toxic metals in chocolate confectionery wrappers

Total no of 48 samples were analyzed from three different brands which are manufactured locally. Brand A consist of 18 samples which includes 6 different products, brand B consist of 18 samples which includes 6 different products and brand C consist of 12 samples which includes 4 different products. Table 4 illustrates the quantified levels of the toxic metals found in wrappers in brands A, B and C.

Table 4: Concentrations of toxic metals in wrappers in brand A B and C

Sample No	*Brand*	*Concentration/ppm*					
		Cr	*Ni*	*As*	*Cd*	*Sb*	*Pb*
1	A	0.78	ND	ND	ND	ND	0.53
2	A	0.76	ND	ND	ND	ND	0.57
3	A	0.80	ND	ND	ND	ND	0.54
4	A	1.90	ND	ND	ND	1.63	0.70
5	A	1.70	ND	ND	ND	2.23	0.71
6	A	1.90	ND	ND	ND	2.41	0.74
7	A	11.70	29.50	ND	ND	ND	4.00
8	A	11.50	30.00	ND	ND	ND	3.00
9	A	11.40	31.00	ND	ND	ND	3.90
10	A	0.44	ND	ND	ND	1.50	ND
11	A	0.45	ND	ND	ND	1.00	ND
12	A	0.44	ND	ND	ND	1.00	ND
13	A	0.70	2.60	ND	ND	ND	1.50
14	A	0.70	2.70	ND	ND	ND	1.70
15	A	0.70	2.60	ND	ND	ND	1.50
16	A	1.20	ND	ND	ND	ND	0.50
17	A	1.40	ND	ND	ND	ND	0.60
18	A	1.10	ND	ND	ND	ND	0.50
19	B	3.52	9.63	ND	ND	1.27	3.50
20	B	4.18	9.00	ND	ND	2.30	4.00
21	B	4.24	9.00	ND	ND	3.10	4.00
22	B	3.61	7.10	ND	ND	3.10	5.62
23	B	3.10	7.10	ND	ND	3.00	5.60
24	B	3.30	7.50	ND	ND	3.10	4.00
25	B	7.03	7.67	ND	ND	27.00	4.50

26	B	7.00	7.10	ND	ND	28.00	4.50
27	B	7.01	7.10	ND	ND	25.00	4.60
28	B	6.51	7.10	ND	ND	7.90	3.33
29	B	6.60	8.00	ND	ND	8.70	3.40
30	B	6.80	7.80	ND	ND	8.80	3.50
31	B	5.52	6.65	ND	ND	11.40	2.09
32	B	4.98	6.62	ND	ND	11.20	1.98
33	B	5.00	6.61	ND	ND	11.10	2.01
34	B	144.40	28.00	ND	ND	ND	4.30
35	B	141.00	32.50	ND	ND	ND	4.80
36	B	146.00	30.30	ND	ND	ND	4.40
37	C	175.00	29.00	ND	ND	ND	5.00
38	C	180.00	36.00	ND	ND	ND	5.60
39	C	186.00	33.50	ND	ND	ND	5.40
40	C	252.00	23.50	ND	ND	ND	6.01
41	C	260.00	22.00	ND	ND	ND	7.06
42	C	258.00	20.50	ND	ND	ND	6.86
43	C	388.00	32.60	ND	ND	ND	5.60
44	C	390.00	37.40	ND	ND	ND	6.30
45	C	395.00	35.40	ND	ND	ND	6.00
46	C	381.00	26.60	ND	ND	ND	4.80
47	C	385.00	25.70	ND	ND	ND	5.10
48	C	388.00	26.20	ND	ND	ND	5.00

ND- Not Detected. ppm- Parts per million.

Table 5 illustrates the average levels of toxic metals detected in chocolate wrappers. Arsenic (As) and Cadmium (Cd) were not detected in chocolate wrappers in brand A B and C. According to the results obtained the highest Cr, Ni and Pb contents were found in brand C chocolate wrappers. These wrappers were Blue, Green, Red, and Yellow/Gold metalized wrappers. Highest Sb content was found in brand B and they were flexible wrappers (Polyethylene and polypropylene films). According to the literature in the production process of thermoplastic polymers, such as polyethylene and polypropylene different catalysts may be used which can contain low levels of heavy metals. The sources for Cd and Pb are impurities originating from inorganic pigments and stabilizers. Antioxidants can contain Ni, while thermal stabilizers can contain Ni, Pb and Sb [13].Cr is used as a pigment in printing inks. This Cr can be present as Cr (III) or Cr (VI).

According to the maximum limits in EU Directive 94/62/EC (amended by 2004/12/EC) on "packaging & packaging waste", the Pb levels in wrappers are below the maximum level of 100ppm. Limits for Ni, Sb and total Cr have not been developed.

The results were statistically analyzed by Non-Parametrically and the significant levels were obtained by Kruskal-Wallis Test. The results exist in a significant difference between the same metal concentrations, in different product wrappers within the same brand at a P value of< 0.05.

Table 5: Average concentration of toxic metals in wrappers

Brand	*Concentration/ppm*					
	Cr	*Ni*	*As*	*Cd*	*Sb*	*Pb*
A	2.75	16.40	ND	ND	1.63	1.40
B	28.32	11.38	ND	ND	10.33	3.90
C	303.17	29.03	ND	ND	ND	5.73

ND- Not Detected, ppm – parts per million

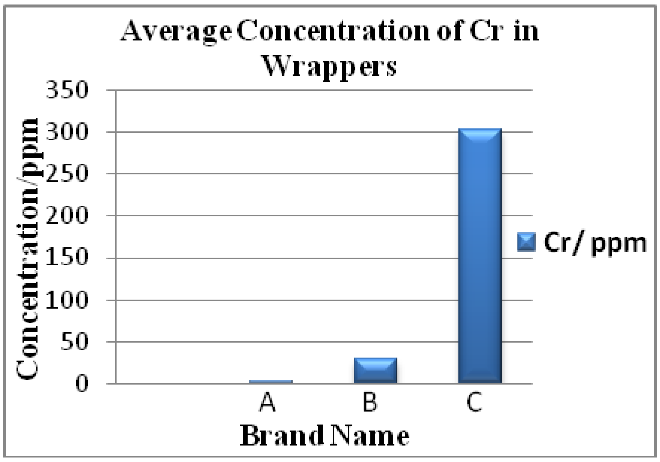


Figure 1: Average Concentration of Cr in wrappers

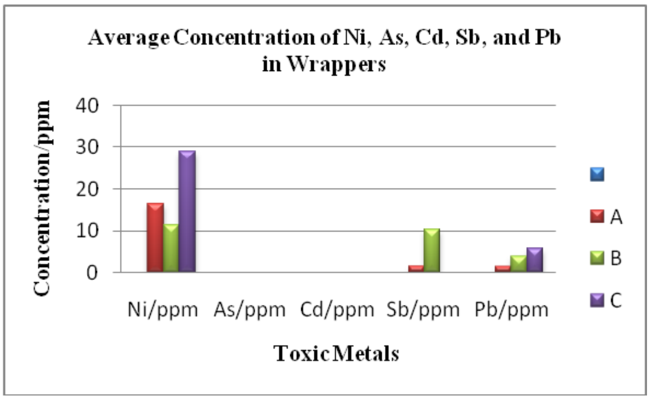


Figure 2: Average Concentration of Ni, As, Cd, Sb, and Pb in brands A, B and C wrappers

4.3 Levels of toxic metals in chocolate confectioneries.

Brand A

Total no of 18 chocolate samples were tested from brand A, and it consist of 6 different products. Table 6 illustrates the concentrations of toxic metals found in brand A chocolates. As, Cd, Sb and Pb were not detected in brand A chocolates. Cr was detected in 6 of 18 samples, which was stored in room temperature, with an average of 0.4ppm**.** The wrappers of these samples were Blue coloured metalized and Black coloured flexible materials.

Brand B

Total no of 18 chocolate samples were tested from brand B, and it consist of 6 different products. Table 7 illustrates the concentrations of Toxic metals found in brand B chocolates. As, Cr, and Cd were not detected Sb was detected in 6 of 18 samples, which was stored under room temperature, with an average of 1.2ppm**.** Pb was detected in 3 of 18 samples, which was stored in both room temperature and refrigerator, with an average of 0.2 ppm. The wrappers of these samples were flexible (Polypropylene) materials. According to FDA the maximum limits for Pb in chocolates is 0.1ppm.Therefore the detected levels are slightly exceeding the maximum limits in 3 samples of brand B chocolates. Statistical data analysis shows that the P value is 0.8454 which is > 0.05.Therefore there is no significant difference between the median Pb concentration of the chocolates stored at the room temperature and the chocolates kept in refrigerator.

Table 6: Concentrations of Toxic metals in brand A chocolates.

		Cr/ ppm			*Ni /ppm*		
Sample no	*Brand*	*CT*	*RT*	*RF*	*CT*	*RT*	*RF*
1	A	ND	ND	ND	0.49	0.6	ND
2	A	ND	ND	ND	0.51	0.61	ND
3	A	ND	ND	ND	0.49	0.6	ND
4	A	ND	0.4	ND	0.96	ND	0.73
5	A	ND	0.42	ND	0.89	ND	0.77
6	A	ND	0.4	ND	0.9	ND	0.72
7	A	ND	0.4	ND	1.8	ND	0.39
8	A	ND	0.38	ND	1.8	ND	0.41
9	A	ND	0.37	ND	1.82	ND	0.34
10	A	ND	ND	ND	ND	ND	ND
11	A	ND	ND	ND	ND	ND	ND
12	A	ND	ND	ND	ND	ND	ND
13	A	ND	ND	ND	ND	0.8	1.17
14	A	ND	ND	ND	ND	0.76	1.23
15	A	ND	ND	ND	ND	0.84	1.27
16	A	ND	ND	ND	1.15	0.63	0.94
17	A	ND	ND	ND	1.25	0.69	0.12
18	A	ND	ND	ND	1.18	0.71	0.98

CT- Control Sample, RT- Room Temperature, RF- Refrigerator

Table 7: Concentrations of Toxic metals in brand B chocolates

		Ni /ppm			*Sb /ppm*			*Pb /ppm*	
Sample no		*CT*	*RT*	*RF*	*CT*	*RT*	*RF*	*CT*	*RT*
19	B	2	ND	ND	ND	1.26	ND	ND	ND
20	B	2.2	ND	ND	ND	1.23	ND	ND	ND
21	B	2	ND	ND	ND	1.28	ND	ND	ND
22	B	2.3	ND	ND	ND	1.14	ND	ND	ND
23	B	2.3	ND	ND	ND	1.18	ND	ND	ND
24	B	2.3	ND	ND	ND	1.13	ND	ND	ND
25	B	ND	0.42	1.4	ND	ND	ND	ND	0.36
26	B	ND	0.46	1.39	ND	ND	ND	ND	0.38
27	B	ND	0.43	1.37	ND	ND	ND	ND	0.35
28	B	ND	ND	0.93	ND	ND	ND	ND	ND
29	B	ND	ND	0.95	ND	ND	ND	ND	ND
30	B	ND	ND	0.98	ND	ND	ND	ND	ND
31	B	0.5	ND	ND	ND	ND	ND	ND	ND
32	B	0.5	ND	ND	ND	ND	ND	ND	ND
33	B	0.5	ND	ND	ND	ND	ND	ND	ND
34	B	ND	1.14	0.71	ND	ND	ND	ND	ND
35	B	ND	1.11	0.75	ND	ND	ND	ND	ND
36	B	ND	1.09	0.74	ND	ND	ND	ND	ND

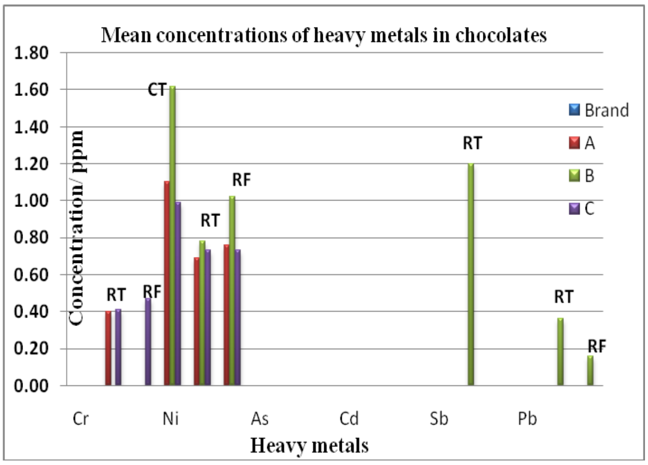
Brand C

Table 8. Illustrates the concentrations of Toxic metals found in brand C chocolates. Total no of 12 chocolate samples were tested, and it consist of 4 different products. As, Cd, Sb and Pb were not detected, Cr was detected in 6 of 12 samples, which was stored in room temperature and refrigerator, with an average of 0.41ppm and 0.47ppm respectively**.** The wrappers of these samples were Red and Green coloured metalized materials and the detected average Cr level in wrappers was 300ppm. The migration is allowed when the wrappers are closely adheres to the chocolate. The obtained P value for Cr was > 0.05. Therefore there is no significant difference between the median Cr concentration of chocolates stored at the room temperature and refrigerator.

Nickel was found in brand A, B and C chocolates in a range of 0.7 – 1ppm. Since the control samples exist in a higher concentration it can conclude that Ni migration has not taken place from the wrapper. According to literature, chocolate is one of the foods with the highest nickel content. The pure cocoa has Ni content up to 8.2-12 ppm in fresh wet weight [2]. Statistical data analysis shows that the P value is 0.2306 which is > 0.05.Therefore there is no significant difference between the median Ni concentration of the chocolates kept at the room temperature and the chocolates kept in refrigerator.

Table 8: Concentrations of Toxic metals in brand C chocolates

		Cr /ppm			*Ni /ppm*		
Sample no	Brand	CT	RT	RF	CT	RT	RF
37	C	ND	ND	ND	1.17	0.4	0.8
38	C	ND	ND	ND	1.19	0.41	0.82
39	C	ND	ND	ND	1.16	0.4	0.84
40	C	ND	0.41	0.53	0.52	1.07	0.8
41	C	ND	0.42	0.51	0.51	1.1	0.83
42	C	ND	0.41	0.53	0.52	1.09	0.79
43	C	ND	0.42	0.41	0.55	ND	ND
44	C	ND	0.42	0.41	0.58	ND	ND
45	C	ND	0.4	0.43	0.52	ND	ND
46	C	ND	ND	ND	1.74	0.69	0.56
47	C	ND	ND	ND	1.72	0.72	0.53
48	C	ND	ND	ND	1.7	0.67	0.62



CT- Control Sample, RT- Room Temperature, RF- Refrigerator

Figure 3: Graphical representation of mean concentrations of toxic metals found in chocolates of brand A, B and C kept in room temperature and refrigerator.

5. Conclusion and Future Work

On the basis of the analyzed results it can be concluded that Cr, Ni, Sb and Pb were detected at higher concentrations in chocolate confectionery wrappers used by the local manufacturers. As and Cd were not detected neither in chocolates nor wrappers. Cr was detected from 2-300ppm in all 48 packages, Ni was detected from 2-30ppm in 36 of 48 packages, Sb was detected from 1-28ppm in 21 of 48 packages and Pb was detected from 0.5ppm-6ppm in 45 of 48 packages. The metal content was generally found to be low in chocolates. Ni was present in a 0.7 – 1ppm range. It was found that Ni was present in most of control samples, in all three brands. Cr, Sb and Pb were present in the chocolates with an average concentration of 0.4ppm, 1ppm, and 0.2ppm respectively. This was shown when the wrapper is closely adheres to the chocolate. As depicted by the statistical analysis it can be concluded that the results obtained by the two storage conditions were not significantly different, but the migration of toxic metals are favorable in room temperature. There are no well defined limits for above analyzed metals in chocolates, except for Pb, Cd, and As. It is necessary to conduct further studies on Speciation between Chromium (VI) and Chromium (III) in Chocolate wrappers to determine the levels of Chromium (VI).

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