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Quantitative Bacterial Examination and Chemical Evaluation of Diet, Club, and Ice-cream Sodas, Soft Drinks

الفحص الجرثومي الكمي والتقييم الكيميائي، لمشروبات الحمية ، وكلوميت أو كلوب ، والآيس كريم صودا، الغازية المُنكهة

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Abstract: Diet, club, and ice-cream sodas are flavoured soft drinks consumed throughout the world, especially in summer seasons. This study has been undertaken to monitor the bacterial and chemical contamination of these national and international branded drinks procured from local markets. The isolated coliforms and microbes were *E. coli, Salmonella spp, Klebsiella spp, Enterobacter spp, Shigella spp and Bacillus cereus.* Diet and club sodas were less contaminated with microorganisms than were ice-cream sodas.

Fifteen trace and toxic elements were identified with an atomic absorption spectrophotometer following the improved ash digestion method. The values of Nickel (Ni), (0.15 mg/L), (Pb (0.28 mg/L), Cadmium (Cd) (0.13 mg/L) and (Al (0.76 mg/L), were higher than the (WHO) recommended limits. The concentrations of (Na, Fe, Pb) and Chromium (Cr) were higher in club sodas than diet and ice-cream sodas and the concentrations of Calcium (Ca), Magnesium (Mg), Potassium (K), Iron (Fe), Zinc (Zn), Aluminum (Al), Barium (Ba), (Co), Cobalt, and Manganese (Mn) in ice-cream sodas were also higher than diet and club sodas. Overall, the ice-cream sodas did not conform to the (WHO) standards allowed for safe ingestion of micro- and macro-metals in various drinks.

Key words: club soda, diet soda, ice-cream soda, microbial contamination, trace metals.

المستخلص: تهدف الدراسة، إلى الفحص الجرثومي الكمي *(Quantitative biacterial examination)*، والتقييم الكيميائي *(Chemical evaluation)* لعينات من مشروبات الحمية *(Diet)* صودا، وكلوميت أو كلوب *(Club)* صودا، والآيس كريم *(Ice-cream)* صودا، الغازية *(Softdrinks)* المُنكهة، المستهلكة عالمياً، وبمعدلات عالية في فصول الصيف، مع إرتفاع درجة الحرارة. تم جمع عينات البحث من السوق المحلي في إسلام أباد، باكستان. أوضحت نتائج الدراسة أن بكتيريا، كوليفورم *Coliforms*، والميكروبيس *Microbes* ، المعزولة، هي من فصيلة أو جنس، سالمونيلا *Salmonella speies*، كليبسيلا *Klebsiella spp* ، وانتيروباكتر *Enterobacter spp*، شيجيلا *Shigella spp* ، إضافةً إلى اسشيريشيا كولى *Escherichia coli*، وبالسيليوس كيريوس . *(Bacillus cereus)* أوضحت الدراسة أيضاً، أن عينة مشروبي الحمية (Diet) صودا، وكلوميت أو كلوب *(Club)* صودا، أقل، أو أخف تلوثاً بالميكروبات المجهرية الدقيقة، مقارنةً مع مشروب الآيس كريم *(Ice-cream)* صودا. تم تعريف عدد (15) أثر وعناصر سامة بخاصية الإمتصاص الذري. وذلك بمقياس سبيكروفوتوميتر *(Spectrophotometer)*، بطريقة الهضم بالرماد *(Ash digestion)*، المُحَسنة. وكانت قيم عناصر (النيكل (Ni)، *0.15* ملجم/لتر)، و(الرصاص (Pb)، *0.28* ملجم/لتر)، (الكادميوم (Cd)، *0.13* ملجم/لتر)، و(الألمنيوم (Al)، *0.76* ملجم/لتر)، أعلى من حدود القيم القياسية لمنظمة الصحة العالمية، (WHO).أما تركيز عناصر، الصوديوم (Na)، الحديد (Fe)، والرصاص (Pb)، والكروميوم (Cr)، كانت هي الأعلى في عينة مشروب الكلوميت أو الكلوب *(Club)* صودا، منها في عينة مشروبي الحمية *(Diet)* صودا، والآيس كريم *(Ice-cream)* صودا. كما أن تركيز عناصر، الكالسيوم (Ca)، والمغنسيوم (Mg)، والبوتاسيوم (K)، والحديد (Fe)، والزنك (Zn)، والألمنيوم (Al)، والباريوم (Ba)، والكوبالت (Co) والمانجنيس .(Mn) هي أعلى تركيزاً في عينة مشروب الآيس كريم *(Ice-crem)* صودا، مقارنةً، مع عينة مشروبي الحمية *(Diet)* صودا، والكلوميت أو كلوب *(Club)* صودا. عليه برهنت الدراسة أن عينة مشروب الآيس كريم *(Ice-cream)* صودا، لا يتطابق مع معايير ومواصفة منظمة الصحة العالمية (WHO)، للوقاية من إحتواء المشروبات الغازية على المعادن والعناصر المجهرية والدقيقة.

كلمات مدخلية: مشروبات غازية، الحمية صودا، كلوميت أو كلوب صودا، آيس كريم صودا، تلوث، معادن، عناصر سامة.

Introduction

Soft drinks are a class of nonalcoholic beverages, usually but not necessarily carbonated, containing a sweetening agent, edible acids and neutral or artificial flavours. These drinks are used more for their social nature, rather than their nutritional quality. Soft drink production begins with the creation of flavoured syrup using a 'closely guarded' company recipe. The syrup is mixed with purified water and then carbonated by adding carbon dioxide gas under pressure. This carbonation creats the 'tingly fizz' that gives soft drinks a refreshing taste. The flavour and taste of soft drinks depend on subtle amounts and combinations of ingredients. Natural flavours are derived from fruits, nuts, berries, roots, herbs and other plant sources. At least 86% of a soft drink is purified water. (Miettinen, *et al.* 1996); (Wattoo, 2000a); (Waqar Ashraf, *et al.* 2000)

Diet drinks are sugar free flavoured soft drinks. These are made by most of the multinational companies, to offer their flavoured drinks to diabetics. Club soda is one of many names for water that has been charged with carbon dioxide, sodium

bicarbonate, and sometimes flavours. It is also referred to as 'soda water', 'carbonated water', 'sparkling water', and 'seltzer water'. Soda water was first bottled in the United States in 1835. Ice-cream soda was born in 1874, at a fair in Philadelphia, when Robert M. Green, a concessionaire, substituted vanilla ice cream after the sweat cream, which was used in his flavoured soft drinks, ran short. The mixture of syrup, carbonated water and ice-cream was an instant hit that allowed Green's profits to increase from $6 to over $600 per day. (Wattoo, 2000a)

Club, ice-cream and diet sodas, packed in attractive glass bottles, are commonly sold in the commercial markets of big cities. Emulsifiers are widely used in many of them, (Kuykendall, *et al.* 1996) and are of synthetic or natural origin. Water used in soft drinks must be soft and free from any appreciable amount of trace metals and infectious organic matter. Low-grade soft drinks or those made from water contaminated with microorganisms, parasites, or chemicals, have caused food borne illness (Khurshid & Qureshi, 1984); (Wasim Yawar & Sohaila Rahman, 1997). 80% of human diseases are water borne. Most untreated water contains large numbers of bacteria and parasites, (Warburton, 1993); (Delves, 1995); (Chatwal, 1997); (Carson, *et al.* 1998); (Daley & Melvin, 1998); (Kratz, *et al.* 1999); (Mariam, *et al.* 2000); (Squier, *et al.* 2001). The number and types of bacteria in water vary depending upon its source (mainly municipal water supplies), its quality and the type of decontamination used. Substandard drinks may cause cholera, typhoid, amoebiasis, helminthiasis, and hepatitis A (Wattoo, *et al.* 2000 b, c, d). In addition, chemical impurities such as heavy metals, could cause adverse health effects due to prolonged exposure. Some chemicals can affect the appearance and taste of these sodas (Bukhari, *et al.* 1987); (Musaiger, 1990); (Kuykendall, *et al.* 1996); (Wasim Yawar and Sohaila Rahman, 1997); (Nikno and Asubiojo, 1997); (Lima, *et al.* 1998). There is no published data on the level of chemicals in soft drinks available in Pakistan.

The main purpose of this study was to determine the chemical and microbial quality of different brands of club, diet and ice-cream sodas sold in local markets of Hyderabad and Karachi. The objectives were to quantify

(1) The microbial contamination.

(2) The chemical levels in several soft drinks.

Materials and Methods

● Reagents and solutions

Standards for Atomic Absorption Spectrophotometer provided by (FLUKA) Company were used. Fresh standards were prepared by diluting stock standards with de-ionized water just before use. Statistical data for standard of elements is given in (Table 1). All the chemicals used were of analytical reagent grade.

Table 1. Statistical Data for Standard of Elements.

Elements	Conc. Range ppm (X)	Absorption range	Statistical calculation y = m x + c		
			m	c	R^2
Calcium	0.0 – 5.0	0.0 – 0.168	0.0329	0.0031	0.996
Magnesium	0.0 – 1.0	0.0 – 0.637	0.641	-0.0033	0.999
Sodium	0.0 – 2.0	0.0 – 1.3	0.645	0.0092	0.999
Potassium	0.0 – 1.0	0.0 – 0.191	0.1898	0.0038	0.998
Iron	0.0 – 1.0	0.0 – 0.091	0.0904	0.0015	0.999
Nickel	0.0 – 1.0	0.0 – 0.063	0.0627	0.0027	0.992
Copper	0.0 – 1.0	0.0 – 0.0865	0.0861	0.0009	0.997
Zinc	0.0 – 1.0	0.0 – 0.197	0.1962	0.0008	0.999
Lead	0.0 – 1.0	0.0 – 0.168	0.0166	-0.0002	0.998
Cadmium	0.0 – 1.0	0.0 – 0.175	0.1753	0.0010	0.999
Aluminium	0.0 – 10.0	0.0 – 0.35	0.0033	-0.0003	0.999
Barium	0.0 – 5.0	0.0 – 30*	6.032	-0.5200	0.998
Chromium	0.0 – 0.25	0.0 – 22*	88.32	-0.3600	0.998
Cobalt	0.0 – 1.0	0.0 – 0.029	0.0293	-0.0004	0.999
Manganese	0.0 – 1.0	0.0 – 0.188	0.1858	0.0030	0.999

Key: * = y is division of recorder at expansion x 5

● Sample treatment

Four to five samples of each brand were collected/purchased, from the local markets of Hyderabad and Karachi, in their original packing. First of all, the samples were analysed for microbiological study. The glass bottles in their original packing were opened individually in a laminar flow chamber under all possible aseptic measures. All subsequent procedures were carried out aseptically. Eosin Methylene Blue Agar (EMBA), media was prepared in an autoclave. The Membrane Filter Technique (MFT) was adopted for all the microbiological studies due to its many desirable features (Buchanan & Gibbons, 1975); (Cappuccino & Sherman, 1987); (States, *et al.* 2000); (Wattoo, 2000a).

In the second step, (CO_2) was removed by transferring the sample to a large flask and shaking, gently at first and then vigorously, while maintaining the temperature of the soft drink sample at 20°–25°C. For determination of sodium, potassium, calcium, and magnesium, one (ml) of degassed sample was diluted with (2N HNO_3) to a volume (100ml). For trace elemental analysis, (100ml) of sample was pre-concentrated twenty times by an ash digestion method (Lima, *et al.* 1998); (Haraguchi, 1999); (Wattoo, *et al.* 2000a). We have improved this method as follows:

(**a**) 100ml of degassed soft drink sample was taken in a flat bottom flask

(**b**) Concentrated (HNO_3) and (H_2SO_4), in equal amounts (1:1) was added.

(**c**) 30% (H_2O_2) was used to enhance the oxidation of interfering organic matter.

(**d**) It was observed that the addition of (H_2O_2) should be proportional to the amount of sugar present in the sample (Wattoo, *et al.* 2000b, c).

● Instrumentation

The microbiological study was made by membrane filter technique in a laminar flow chamber (model 4–303–30–362, Liberty Industries, East Berlin, CT, USA) under all possible aseptic measures. A Colony Counter (GallenKamp) and autoclave (LS–2D, Rexall Industries, Taiwan) were used. Glass micro-fiber filters (GF/F), of Whatman were used (Buchanan & Gibbons, (1975); (Cuppuccino & Sherman, 1987).

Trace elemental analysis was carried out using an atomic absorption spectrometer (AAS), (Hitachi model 180–50) equipped with a deuterium background corrector. The operating parameters for working elements were set as recommended by the manufacturer. Hollow cathode lamps of Mitorika brand were used for the elements of interest. Samples were atomized in an acetylene–nitrous oxide flame for the determination of aluminium and barium. For the remaining an air–acetylene flame was used. Prior to analysis, the instrument was calibrated according to the manufacturer's recommendations.

Results and Discussion

(1) Microbiological study

Microbiological potability standards for soft drinks in most developed countries, rely on the detection of total coliforms and *E.coli.* as markers for human pathogens (Miettinen, *et al.* 1998); (Wattoo, *et al.* 2000a). The U.S. Environmental Protection Agency (USEPA), suggests a maximum contamination level for total coliforms and *E.coli.* as <5% (Positive) and (0.00) (organisms/100ml), respectively (HACH-catalogue 1999). Data in Table (2) show that coliforms ranged from <1.0 to 43/100ml. Only 2 of the 10 samples were free of potential food borne pathogens (Table 2). Overall, ice-cream sodas had more potential food borne pathogens than did diet and club sodas. *E.coli.* is a normal inhabitant of the intestinal tract of humans and other warm blooded animals (Kratz, *et al.* 1999); (AbdulKarem & Hassan, 2000); (Marium, *et al.* 2000). Its presence in (DS-3, ICS-8 and ICS-10) indicates that the water used to make these drinks was contaminated with fecal matter. *Salmonella species* were found in 3 of the 4 ice-cream sodas (ICS-8-10) and in one each of diet (DS-3) and club (CS-6) sodas (Table 2). *Salmonella species* are bacteria that produce typhoid fever, enteritis and food borne illness (Buchanan & Gibbons, 1975); (Cappuccino & Sherman, 1987).

All except two samples and all the ice-cream sodas have *Klebsiella species* (Table 2). *Klebsiella* species cause urinary and respiratory infections (Buchanan, *et al.* 1975); (Cappuccino, *et al.* 1987). Their presence suggests that contaminated water was used. *Enterobacter species* were only found in (ICS-9) at 6 to 8 cells/100ml (See, table 2) and are common in human and animal feces, sewage water, and soil (Buchanan, *et al.* 1975); (Cappuccino, *et al.* 1987). *Shigella species* were isolated from 7 to 10 samples with (ICS-7) having a range from 15 to 18 *Shigella*/100ml. *Shigella species* are common in the intestines of humans and primates and produce dysentery and food borne gastroenteritis

Table 2. Isolated coliforms in Diet, Club and Ice-cream Sodas soft drinks.

(Organism / 100 ml.)

S. Code.	*E. coli*	*Salmonella spp*	*Klebsiella spp*	*Entero-bacter spp*	*Shigella spp spp*	*Bacillus-cereus*	*Fungus*	Total Coliforms
D S – 1	--	--	2 – 3	--	2 – 4	--	2 – 3	8 – 10
D S – 2	--	--	--	--	--	--	--	--
D S – 3	2 – 3	3 – 4	6 – 7	--	--	--	--	10 – 12
C S – 4	--	--	7 – 9	--	9 – 10	9 – 11	--	25 – 27
C S – 5	--	--	--	--	--	--	--	--
C S – 6	--	3 – 5	6 – 7	--	7 – 8	4 – 5	--	20 – 25
ICS – 7	--	--	16 – 18	--	15 – 18	--	--	35 – 39
ICS – 8	2 – 3	3 – 5	2 – 3	--	2 – 4	--	2 – 3	11 – 18
ICS – 9	--	2 – 3	4 – 6	6 – 8	3 – 5	2 – 3	--	17 – 25
ICS – 10	2 – 3	2 – 3	10 – 15	--	10 – 12	6 – 10	--	30 – 43

U. S. Environmental Protection Agency Guidelines for drinking water i.e. Max. Contamination Level
1= Total Coliforms = < 5% Positive. (Organisms/100 ml) Samples/month.
2= *E.coli* = (0.00) (Organisms/100 ml)

(Buchanan, *et al.* 1975), (Cappuccino, *et al.* 1987). Another bacterium that causes food borne illness, *Bacillus cereus* (Kratz, *et al.* 1999), was observed in four samples with (CS-4 and ICS-10) having 6 to 11 cells/100 ml. Only (DS-1 and ICS-8) had fungal contamination.

(**2**) Trace elemental study

An element is considered to be toxic if it binds at non-binding centers, causes precipitation of metals of metalloenzymes or replaces essential elements of the same charge or shape in the molecule and enzymes (Wattoo, *et al.* 2000c). The human body has an almost zero tolerance limit for known toxic elements; therefore, their amounts in soft drinks need periodic monitoring.

All the samples have calcium and magnesium levels (Table 3) within the (WHO) safe limits; i.e. below 100mg/L and 50mg/L respectively. An excessive concentration of calcium and magnesium (hardness) is associated with heavy stomach disorder and imparts a laxative nature to the beverages. However, the presence of calcium within the permissible limits is useful for the body as supplement of diet (Sadiq & Hussain, 1997); (Abdulrahman, 1997); (Wattoo, *et al.* 2000d).

Four samples were observed with higher values of potassium than the legal recommendations. The observed higher values were due to using cheaper quality glass bottles. The highest sodium value found was 152.98mg/L in (CS-5) sample. There is evidence that high intake of sodium may contribute to the development of hypertension in persons who are susceptible. Hypertension is one of the main public health problems in Pakistan. Attention, therefore, should be given to the sodium level of these beverages in the country. Persons with hypertension need a sodium-restricted diet. The use of these beverages may have a significant role in elevating the blood pressure of these persons. Both play an important role in establishing the electrolytic balance in the human body (Bukhari, *et al.* 1987); (Musaiger, 1990).

The highest value of iron (Table 3) observed was 1.44mg/L in (CS-6) which exceeds the permissible limit (0.3 mg/L). Iron is essential to the human body but its intake through drinking water is an insignificant part of the body requirement. The permissible limit placed on this metal has no health significance but it renders water unsuitable for human hygiene and other human requirements. In the present study variation in the concentration of iron may be attributed to local contamination due to the corrosion in the pipelines or plumbing system. (Bukhari, *et al.* 1987); (Abdulrahman, 1997).

All the samples (Table 4) have nickel levels above recommendations (0.02 mg/L). Nickel compounds are found in many ores and minerals and are toxic in the conventional sense. Deficiency of nickel may lead to health problems such as dermatitis and deformities of bones, while excess intake of nickel may cause lung cancer and myocardial infarction. Corrosion of pipes appears to be responsible for the wide variations in nickel and copper concentrations of the beverages (Bukhari, *et al.* 1987); (Abdulrahman, 1997); (Sadiq & Hussain, 1997).

Table 3. Concentration of Trace and Toxic Elements in Diet, Club and Ice-cream Sodas soft drinks.
(mg / liter)

S. Code.	Ca	Mg	Na	K	Fe
W H O	100	30 – 50	200	12	0.3
D S – 1	17.36 - 18.82	6.02 - 6.16	62.50 - 62.83	28.88 - 29.89	0.85 - 0.92
D S – 2	7.15 - 8.61	0.28 - 0.41	104.97 - 106.63	5.87 - 6.37	0.91 - 0.98
D S – 3	17.36 - 18.82	8.43 - 8.69	100.84 - 106.13	5.36 - 5.87	0.51 - 0.59
C S – 4	23.20 - 24.67	2.42 - 2.68	104.14 - 107.95	12.44 - 13.20	0.49 - 0.61
C S – 5	14.44 - 15.91	4.55 - 4.69	141.07 - 152.98	5.11 - 5.36	0.99 - 1.44
C S – 6	18.71 - 20.19	3.35 - 5.12	138.78 - 145.15	8.34 - 10.15	0.55 - 1.00
ICS – 7	43.62 - 45.08	5.62 - 5.89	94.40 - 95.38	14.47 - 14.72	1.13 - 1.23
ICS – 8	29.03 - 30.49	8.69 - 8.96	70.60 - 73.90	10.42 - 11.18	1.01 - 1.10
ICS – 9	35.15 - 37.18	6.42 - 7.12	98.15 - 99.05	14.28 - 14.85	1.08 - 1.25
ICS – 10	44.23 - 45.15	8.85 - 9.00	72.88 - 74.36	11.08 - 11.25	1.00 - 1.05

DS = Diet Soda Drinks, CS = Club Soda Drinks, ICS = Ice Cream Soda Drinks.
(WHO) = World Health Organization standard values (HACH – Products for Analysis, 1999, page # 16)

In the present study, all the samples (Table 4) had copper values within the safety limits. Copper is not a significant constituent in natural waters; however, it is introduced by dissolution of copper from brass and copper pipes and by the use of copper sulphate as an algaecide in reservoirs. Copper is of physiological importance as a supplement to iron for hemoglobin regeneration and is an essential constituent of cells. Excess of copper is known to affect the gastrointestinal system and to cause hemochromatolysis (Chatwal, 1997); (Wattoo, *et al.* 2000d).

In all the samples (Table 4) the level of zinc was found within the recommended limits. Zinc is rarely present in natural waters and the most probable reason of zinc increase in the product might be its contribution from the zinc galvanized pipes and plumbing material within the plant and from chemicals used in the treatment process. Zinc is a normal constituent of the human body and no significant health hazard is observed up to 40ppm, though the permissible limit is 3mg/L. (Bukhari, *et al.* 1987); (Waqar Ashraf, *et al.* 2000).

The maximum allowable limits of lead and cadmium prescribed by (WHO) are 0.01 and 0.003mg/L respectively. The amounts of lead and cadmium (Table 4) were observed, surprisingly, higher than the legal limits, which indicates the extent of pollution in soft drinks sold in markets. Lead and cadmium are the most toxic elements. The (PVE) pipes contained about 1% lead, which gradually leached to the plant water, thus increasing lead concentrations in the beverages (Sadiq & Hussain, 1997); (Lima, *et al.* 1998); (Waqar Ashraf, *et al.* 2000).

Table 4. Concentration of Trace and Toxic Elements in Diet, Club and Ice-cream Sodas soft drinks.
(mg / liter)

S. Code.	Ni	Cu	Zn	Pb	Cd
W H O	0.02	1 – 2	3	0.01	0.003
D S – 1	0.11 - 0.13	0.12 – 0.15	0.09 - 0.10	0.17 - 0.20	0.05 - 0.09
D S – 2	0.09 - 0.11	0.01 – 0.02	0.07 - 0.08	0.15 - 0.19	0.05 - 0.08
D S – 3	0.10 - 0.11	0.02 – 0.03	0.09 - 0.10	0.13 - 0.18	0.04 - 0.09
C S – 4	0.03 - 0.05	0.01 – 0.02	0.17 - 0.20	0.17 - 0.25	0.03 - 0.06
C S – 5	0.11 - 0.12	0.02 – 0.03	0.01 - 0.02	0.19 - 0.28	0.05 - 0.09
C S – 6	0.08 - 0.14	0.02 – 0.04	0.15 - 0.19	0.15 - 0.25	0.04 - 0.07
ICS – 7	0.08 - 0.09	0.02 – 0.03	0.09 - 0.95	0.14 - 0.19	0.04 - 0.07
ICS – 8	0.10 - 0.11	0.02 – 0.03	0.01 - 0.02	0.17 - 0.19	0.05 - 0.08
ICS – 9	0.07 - 0.12	0.01 – 0.02	1.00 - 1.05	0.17 - 0.21	0.05 - 0.08
ICS – 10	0.13 - 0.15	0.02 – 0.03	0.08 - 0.20	0.20 - 0.22	0.08 - 0.13

DS = Diet Soda Drinks, CS = Club Soda Drinks, ICS = Ice Cream Soda Drinks.
(WHO) = World Health Organization standard values (HACH – Products for Analysis, 1999, page # 16)

Aluminium is abundant in nature and it is probable that the biological systems have evolved in its presence. There are increasing and compelling evidences that aluminium is toxic to the brain and skeletal system (Krishnan, *et al.* 1987). All the samples (Table 5) showed values higher than the legal recommended value (0.2mg/L) indicating that soft drinks producing companies are using Alum for the purification of their raw water rather to use standard water purifying methods.

(WHO) standards for barium and chromium are 0.7 and 0.05mg/L respectively. Chromium is a known toxic element (Kuykendall, *et al.* 1996), (De Flora, *et al.* 1997). However, both elements were found within the recommendations.

The levels of cobalt, in all samples, ranged from 0.7mg/L to 0.35mg/L. Cobalt is an essential trace element and nutritional requirement for cobalt is 0.3 – 4.0mg/day. Deficiency of cobalt results in hematological disorders, especially pernicious anaemia, thalassemia and sickle cell anaemia whereas its toxic effects lead to polycythemia (Bukhari, *et al.* 1987). (WHO) has no recommendation for cobalt.

(WHO) standards for manganese are 0.1– 0.5mg/L and samples of the soft drinks (Table 5) were found in the range of 0.02mg/L to 0.38mg/L. Manganese, an essential trace element, has a daily nutritional requirement of about 50μg/Kg of body weight. Manganese plays an important role in bone mineralization, protein and energy metabolism, and metabolic regulation. At high level of exposure, manganese can be associated with a Parkinson-like disease and some reproductive effects that include impotence and decreased fertility amongst men. Manganese usually, is found together with iron and its presence can prove to a nuisance in water supplies, can affect the flavour and colour of food and water and can also react with tannins present in beverages to form a black sludge affecting both the taste and appearance and formation of manganese bacteria in water. Objectionable taste of beverages can occur at concentrations greater than 0.1mg/L. The ores of manganese are very common in water supply lines. Chronic manganese poisoning affects the central nervous system (Bruce, 2002); (Wattoo, *et al.* 2000b, c, d).

Conclusion

It is evident from this study that the levels of potassium, iron, nickel, cadmium, lead and aluminium are above recommended values set by (WHO). Moreover, most of the samples also have high levels of *E.coli* and other microorganisms. This is due to the fact that the beverage industry is not taking care about very important starting material, water. Most of the local industries have old and rusted machinery and there is no check and balance in their plants for quality products.

The present study can be used as base line by health management authorities of Pakistan. In this connection an effective program to control the chemical and biological quality of these drinks is highly recommended. Health education should also focus on the types and quality of soft drinks for drinking purposes, in order to educate the public on the health effects of chemicals and biological constituents present in the soda drinks.

Table 5. Concentration of Trace and Toxic Elements in Diet, Club and Ice-cream Sodas soft drinks.
(mg / liter)

S. Code	Al	Ba	Cr	Co	Mn
W H O	0.2	0.7	0.05	NS	0.1 – 0.5
D S – 1	0.20 - 0.38	0.13 - 0.27	0.017 - 0.022	0.22 - 0.27	0.02 - 0.07
D S – 2	0.20 - 0.35	0.13 - 0.19	0.006 - 0.009	0.22 - 0.25	0.02 - 0.05
D S – 3	0.32 - 0.47	0.13 - 0.19	0.006 - 0.008	0.17 - 0.20	0.02 - 0.06
C S – 4	0.38 - 0.53	0.13 - 0.27	0.009 - 0.012	0.07 - 0.15	0.04 - 0.08
C S – 5	0.56 - 0.76	0.13 - 0.19	0.011 - 0.017	0.22 - 0.26	0.02 - 0.07
C S – 6	0.41 - 0.53	0.15 - 0.25	0.010 - 0.014	0.18 - 0.24	0.03 - 0.08
ICS – 7	0.44 - 0.58	0.13 - 0.27	0.004 - 0.006	0.18 - 0.26	0.13 - 0.38
ICS – 8	0.50 - 0.67	0.31 - 0.57	0.006 - 0.008	0.20 - 0.28	0.02 - 0.05
ICS – 9	0.56 - 0.60	0.25 - 0.31	0.005 - 0.006	0.27 - 0.35	0.19 - 0.35
ICS – 10	0.65 - 0.69	0.48 - 0.67	0.007 - 0.008	0.25 - 0.34	0.03 - 0.08

DS = Diet Soda Drinks, **CS** = Club Soda Drinks, **ICS** = Ice Cream Soda Drinks.
(WHO) = World Health Organization standard values (HACH – Products for Analysis, 1999, page # 16)

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