



## Environmental Awareness :

### Bacterial contamination of Hand- made Beverage

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## Environmental Awareness : Bacterial contamination of hand made beverage .

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### مستخلص البحث :

تهدف هذه الدراسة لمعرفة مدي تلوث المشروبات المصنعة يدوياً الباردة التي تباع خارج المنزل بالمحلات العامة في الأسواق ومواقف المواصلات "النقل" العامة . ومدي فهم البائع لكيفية إعداد وتقديم المشروبات للمستهلك. كما تهدف أيضاً لمعرفة فهم المشتري لخطورة أطعمة الشوارع على صحة الفرد .

استخدم في البحث الاستبانة والتحليل البكتيري لمعرفة بكتريا القولون البرازية بالمشروبات كأداة للبحث لجمع المعلومات المتعلقة بالدراسة .

وقد تم حصر 300 محلاً لبيع المشروبات بمواقف الحافلات لخمس محليات على النحو التالي:

- (1) السوق العربي \_ الخرطوم
- (2) السوق الشعبي \_ الخرطوم
- (3) السوق الشعبي \_ أم درمان
- (4) المحطة الوسطي \_ الخرطوم بحري
- (5) سوق الشهداء \_ أم درمان

اختير من هذه المحلات 150 محلاً لملء الاستبيان وأخذ عينات من المياه والثلج المستعملين لصنع المشروبات. وتم أيضاً أخذ عينات لثلاثة أنواع من العصائر هي ( المانجو و الجافة و البرتقال ). وبعد رصد النتائج النهائية لمعلومات الاستبيان بواسطة الحاسوب واستنتاج النسب المئوية لمعرفة الدلالات الإحصائية للفروض، توصلت الدراسة للنتائج التالية :

\_ يوجد هناك تلوث بكتيري بنسبة 70% بالنسبة للمياه المستعملة للمشروبات و88% بالنسبة لعصير البرتقال و100% بالنسبة لمشروب المانجو والجافة والثلج. وهذا التلوث ناتج من عدم نظافة وتدريب العاملين في مجال الأغذية وصحة البيئة وتلوث البيئة المحيطة بمحلات بيع وإعداد المشروبات الباردة . كما أن نقص دورات المياه قد يزيد من تلوث البيئة المحيطة.

يدرك المشتري مدى خطورة استعمال المشروبات التي تعد وتباع خارج المنزل واستعمالها بكثرة للضرورة نسبة لارتفاع درجة حرارة الجو. وعلى ضوء هذه النتائج خلصت الدراسة إلى وضع عدد من التوصيات من أجل تقديم مشروبات خالية من التلوث.

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## **Environmental Awareness : Bacterial contamination of hand made beverage .**

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### **Abstract:**

Processed street cold beverage for the public round bus terminals in localities in Khartoum State were examined for the MPN Coliforms and faecal Coliforms / 100ml

There was faecal contamination of 70%for water used for the processing of beverage.88%of orange beverage ,100%of mango and guava beverage & ice is used as constituent of cold beverage.

Results of the questionnaire, showed that the unhygienic environment ,the untrained cold beverage vendors with bad habits and the shortage of public water cycles lead to the contamination of street cold beverages.

Food consumers were found to prefer house cold beverages to street cold beverage, but sometimes they find themselves in need to use street cold beverages especially during the hot weather.

The financial limitations of the public health authorities made them unable to execute their responsibility towards cold beverage and food handlers as it should be done .

### **Introduction:**

Sudan has a hot climate so there is a need to drink cold beverages whenever most people are away from their homes. Hand processed cold beverages made from seasonal fruits and sold in the crowded areas are usually preferred to the bottled soda drinks "Pepsi cola" because they believe that they are more cheaper and more nutritious. However, they are usually prepared by unhygienic vendors in unhygienic conditions, and usually avoided by consumers aware of general hygiene.

### **The Objectives of this study are:**

- To find out whether street cold beverages are contaminated with pathogenic bacteria...

To prevent health hazard that is associated with bacterial contamination by environmental awareness.

To find out the consequences of bacterial contamination and \or bacterial growth during processing, handling and storage in unhygienic conditions.

### **Materials and Methods:**

#### **Traditional processing of making cold beverages :**

People who are making cold beverages prepare them in a quick way according to consumers demand. They cut the fruits into slices and put them in containers inside the refrigerators or deep freezer as well as water. In most cases, they use plastic containers. Some of them prepare sugar in small buckets, in order to be ready to add to the mixture.

Processing of cold beverages is as follows: first vendors take some fruits by their hands, and use a small bowl to take water, They put them to be mixed together beside sugar in the blender, then sieved in a big containers and put ice.

### **Study area:**

A survey was carried out in Omdurman, Khartoum and Khartoum North around bus terminals in places of purchasing cold beverages. Also surveys were done to the ministry of health.

The study targeted places included that sell different kinds of hand made street cold beverages in the following bus terminals :

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El Souk El Arabi ,Kartoum .  
El Muhata ElAusta , Khartoum North .  
El Souk El Shabi ,Omdurman .  
El Souk El Shabi , Khartoum .  
Souk El Shouhada , Omdurman .

The areas are chosen to reach the people of different socio-economic situation and different ages, who use street cold beverages. The study samples were collected in the period between November 2007-October 2008 .

**Sampling:**

**Samples used in this study were as follows:**

25 samples of ice;100water,50 guava,50 mango and 50 orange beverage. All these kinds of cold beverages were made from fresh fruits Questionnaire forms designed for this study were distributed to 150 beverage handlers ,150 food consumers and 5 health officers .

All water and beverage samples were subjected to bacteriological testing to detect faecal coliforms and count the viable bacteria.

Labeled sterile vials with screw covers were used and 100 ml of each sample were transferred in every vial. Samples were delivered in the vials by the help of the beverage handlers with their cups that they use for the delivery of the cold beverages to the consumers .

All samples were placed in ice in thermo-flask immediately after collection then taken to the laboratory . The time of collection was between 08.30 to10.00 in the morning. Seven to 10 samples were cultured within 3-4 hours after collection .

**According to Frazier,(1967),the cultures Media Used for the Detection of the Coliform bacteria were:**

**\*-Lactose Broth :**

It consists of 3g-extract meat ,5g.peptone,and 5g.-lactose.All these constituents were per liter. The medium was used for the presumptive test for the detection of Coliform bacteria. Sterilized at 121 °C for 20 min.

**\*-Eosin-Methylene-Blue Agar(EMB):**

It consists of 10g.peptons,5g. lactose,5g.sucrose,2g K<sub>2</sub> HPO<sub>4</sub>, 4g. Eosin, 0,06g.,Methylene and 15g.,Agar.

The medium was prepared by dissolving 28g.of EMB powder in one liter of distilled water. The pH of the medium was adjusted to 7.5 . The medium was used for the completed test .Sterilized at 121°C. for 20 min.,

**\*-Nutrients Agar :**

It composed of beef extract, peptones , sodium ,chloride, distilled water & sugar ( 1-1.5%) . The medium was used the completed test (George & Keith,1966).Sterilized at 121 °C . for 20 min.,.

**\*-Brilliant Green Bile Lactose broth (B.G.B.):**

It consisted of 10g.peptones, 20g.OX bile,10g lactose, 13 ml Brilliant green ,0.1aqueous solution and 1000 ml distilled water .The pH., was adjusted to 7.4 and sterilized at 121°C for 20 min.,

**\*- Sterilization:**

Petri-dishes & graduated pipettes were sterilized in the hot air oven at 160°C for one hr. Tubes, vial , bottles & other glasses containers were sterilized in the autoclaves at 15|b|n2 for 15 min., Including needless sterilized by flaming after every use.

**\*- Preparation of Buffer Dilution:**

Thirty four grams of KH<sub>2</sub> PO<sub>4</sub> were dissolved in 500ml distilled water .Of 1.25 ml of this stock solution 1.25ml were added to one liter distilled water and disposed in amounts of 99 ml in dilution bottles and sterilized at 121°C for 20 min.

**\*- Preparation of Serial Dilution :**

This was carried according to (SMEW)the Standard Method for the Examination of Water & Waste water ,(1975.),one ml of the sample in10-2,10-3 and 10-4, dilutions were transferred respectively after shaking several times with hands to sterile Petri dishes for each dilution in duplication .10 ml Of melted plate count agar at a temperature 44°C 10 ml were poured into the previous dishes . Dishes were then thoroughly mixed to distribute the sample throughout the medium . This was done by gentle moving the dishes to and fro .clock and anti-clock wise .

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Dishes were left to solidify and incubated inverted at 37°C for 48hrs.,,. Plates with 30-300 colonies were counted

#### **Presumptive Coliform Test :**

The multiple tube fermentation technique was performed ,Hobbs and Gilbert(1982). Determination of MPN. Coliform bacteria were carried out. Incubation was carried out in the following manner were according to the standard method to the examination water and wastewater.(1975)

- To each of 3 double strength lactose broth tubes,10ml of the original samples were added .

- To each of 3 single strength lactose broth tubes, 1 ml of the original sample was added.

- To each of 3 single-strength lactose broth tubes, 10 ml of the dilution were added.

All tubes were incubated at 37°C for 48 hr., for the observation of gas production .

#### **Confirmed Total Coliforms Test:**

All tubes of the two highest dilutions showing gas formation in 24 hr. were submitted to the confirmed test using Brilliant Green Bile Broth ( BGB)fermentation tubes.

All tubes of all dilution in which gas was produced only at the end of 48 hrs., ,were submitted to the confirmed test .

The MPN. of Coliforms were calculated according to the Macrady's tubes .

#### **Faecal Coliform Test:**

Of each confirmed positive tube 3 full loop were sub-cultured into Brilliant Green Bile Lactose Broth and incubated at 44.5°C for 24hr. hour.,. Tube showing any amount of gas production were considered positive . Tubes showing positive reaction at 44.5°C were streaked on EMB agar(SMEWW) .

#### **\*Staining Method :**

The fixed slide was flooded with methyl violet solution for 30 seconds then washed with tap water and flooded with iodine solution, for 1minute and washed with tap water The film was decolorized using ethyl



alcohol for 30 seconds , washed with tap water and counterstained with 0.5% safranin solution for 10 seconds ,then washed with water, blotted to dry and seen under oil immersion objective.

**\*-Analysis of Data :**

- Questionnaires were compared by percentage value.
- The result of bacterial analysis was analyzed by computer statistical package (SPSS).

**Result and Discussion :**

Gender were,67.7 % of food handlers were males while 23.3 % were females . All of them were traditional experts .

To have females to work in this kind of work was better than males because usually females have well idea about food preparation, processing and handling than males (Gamman and Sherrington,1990). .El Zubeir (1986),stated that all food-handlers should have an elementary knowledge and understanding of food hygiene , processing and handling .For the age of food handlers who answered the questionnaire, it was found that more than half of respondents age range between ,20-25 years ,making 58% and 22% of them range between 25- 30 year . that means the age of most food handlers was between 35-45, which represents 14% of the whole cold beverage handlers . Training young handlers on processing of cold beverage and food hygiene well effect their behavior .

Educational level of cold beverage handlers showed that 9.3 % were illiterate, 17.3% were of secondary educational level , and 113.3% were of post- secondary educational level .

Educated cold beverage handlers were supposed to be of higher level of awareness than illiterate ones concerning food safety and quality .This agree of Abdussalam, (1987), who stated that health education is the key to ensure that food handlers and consumers receive the basic knowledge of food safety .The residence of 76.7% of cold beverage handlers was in town while the residence of 23.3% of them was round the town. Cold beverage handlers settled round the town were found in shanty towns or in towns that have no complete services such as the

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absence of electricity and tap water , the absence of facilities for waste disposal.

Cold beverage handlers in the town are supposed to be more educated than those round the town . They have more chance to see programs related to food hygiene in television and to read journals and magazines related to this purpose more than cold those round town .

For the presence of license 61.3% of cold beverage handlers have renewed license . 10.7% have un-renewed license and 28% have no license for the place to purchase cold beverage .

Gamma and Sherrington (1990), El Zubeir,(1986), FAO,and WHO,(1977), stated that the place of purchasing all kinds of food should be of adequate size , round construction and easily to be repaired and secured from infestation by rats, mice and insects. Health authorities who visit the place of purchasing cold beverage and food in general before issuing the license should secure the suitability of the place such as the observation of construction, ventilation of the place and the area around in order to give the license .

#### **Medical Examination for producing Health Card:**

Medical examination was done for all cold beverage handlers, 100% produced health cards by health authorities . As the result of medical examination ,health card was given to healthy cold beverage handlers to certify that they were free from diseases which will be transmitted through foods . Those who have a healthy card can work in public places for food preparation , processing and handling FAO.\ WHO.,(1977 ).

For the kind of medical examination ,69.4% of cold beverage handlers practiced physical medical examination while 30.6%of them practiced oral medical examination .

Medical examination is reviewed every year for all cold beverage handlers 100 % .. .

Routine medical examination and laboratory screening of cold beverage handlers is very important for their health ,(FAO \ WHO,1977) .It is essential that every place for purchasing cold beverage should employ people with health card to ensure their health and protect consumers from diseases transmitted through water and cold beverage.

Medical examination for 30.6%of cold beverage handlers was done by an oral medical examination . This case might create a problem of transmission of diseases from cold beverage handlers , who carry pathogenic microorganisms to a wide range of consumers

Frazier,(1967),El Zubeir,(1994), stated that any person engaged in handling food should not handle food if suffers from any disease such as diarrhea and vomiting . Medical examination should be carried out practically to ensure that cold beverage handlers do not suffer any diseases .

#### **The Kinds of Equipment Used for Food Handling :**

26.7% of the surveyed places use glass equipment for purchasing cold beverage , 24% use aluminum equipments , 10% use steel equipment and 3.3%use plastic equipment for handling cold beverages . The health authority permit the use of all kinds of cups that can be washed easily such as glasses, aluminum and steel. This is in agreement with El Zubeir (1999) who suggested not to, use plastic cups in handling cold beverage because they harbor dirt and allow the growth of bacterial pathogens . For the washing of equipment 77.3% washed the cups with soap and water while 22.7% of them wash the cups with water only .

El Zubeir (1999, 2006) stated that the use of detergents with water can penetrate and carry away residues that have hardened on food equipment .

#### **Beverages preparation waste removal :**

**Solid waste of fruits used were disposed in containers,18.7%used closed ones while 62.7 used open ones . .**

For duration of waste removal , and 70.5 % of places dispose their solid waste during one day, and 29.5% of them remove the solid waste in less than one day .

According to Banwart,(1970),FAO and WHO.(1977), and El Zubeir, (1999),Gamman , and Sherrington,(1990), the waste must be stored outside the food preparation area in suitable bin covered with lids . Bins should be emptied regularly .Usually waste attracts flies therefore ,the use of closed container for waste , prevents the breeding and concentration of the flies that lead to food contamination. It is very important to remove the waste as soon as possible . For protection of beverages from flies ,72.7% of beverages covered against flies while 27.3% of them leave the beverages uncovered .

To overcome the problem of flies, 53.3% of places used smoke (Backor) 10 .7 % used insecticides,14% used smoking and insecticides and 22% used nothing .

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Godman , et-al (1981),El Zubeir (1999), realized that diseases i.e intestinal diseases are spread by flies due to their feeding habits .

### **Bacterial Diseases Expected to be found in Cold beverage Handlers:**

Of cold beverages handlers who answered the questionnaire 2% suffered from typhoid fever before , 8% of them suffered from diarrhea or vomiting or both within 3 days. The rest cold beverage handlers had no diseases to be transmitted to cold beverage consumers. .

Hobbs and Gilbert ,(1982),El Zubeir (1986),stated that dangerous microorganisms present in or on the food handlers body can grow and multiply during handling and can be transferred to the food or surfaces that come in contact with foods .Thus food handlers when infected or carrying pathogenic microorganisms are serious sources for food-borne diseases .

### **Tobacco used :**

44% of cold beverage handlers who answered the questionnaire use refreshers during the work .from them 31.87 use Saud,43.37 use Cigarettes and 24.2% of them use both . FAO,(1984),FAO,(1996),El Zubeir,(1999),stated that ,any behavior, which could result in contamination of food, should be prohibited in food handling area. Smoking and Saud involve contact with hand & mouth which might be responsible for the spread of bacteria like : Staphylococcus aureus .

Questionnaire results showed that the hygienic condition of food handlers was found to be good for 42% ,acceptable for 32.7% and bad for 25.3% .Every person engaged in food handling area should maintain a high degree of personal cleanliness and wear suitable protective clothing. Protective clothing should be clean and it should cover all parts of the body liable to contaminate food. .

### **\* The presence of Toilet & Lanterns in Cold Beverage purchasing Areas:**

#### **Habit after going to Lanterns**

22%of the places purchasing cold beverage have their own toilets while 78% do not have. It was clear that there is shortage in public water cycles which lead to contamination of the environment.

FAO,(1995) , stated that human excreta is the principle source of pathogenic organisms causing many communicable diseases particularly enteric diseases.

58.7% of cold beverage handlers wash their hands after using toilets with soap and water. Gamman and Sherrington , (1990), stated that food handlers washed their hands regularly with soap and water especially after using toilets.

### **Consumers knowledge about Street Cold Beverages :**

All cold beverage consumers,100% who answered the questionnaire have knowledge about the danger arising from street food .92%of the consumers use street cold beverage for the need to drink because of the hot climate while 8%use street cold beverage without reasoning . According to ElZubeir,(1986),the food stuffs utilized during the preparation should not be contaminated , according to food and health regulations . All places for purchasing cold beverages must have licenses certified by health authorities . Consumers must be aware that food is healthy and differentiate between authorized and unauthorized places for purchasing foods.

According to FAO / WHO,(1977), street foods being available at a low price are served to a large number of consumers of different ages and occupations .This is usually due to the economic situation and the limited income of the people . All respondents of cold beverage consumers 100% prefer house prepared cold beverages than streets ones. The preference of house cold beverage reflects the awareness of consumers about food hygiene practices . The appearances and the personal hygienic practices of the employees are also very important as the way food is handled and the way equipment and utensils are cleaned .

### **\* The kind of Enteric Diseases of food consumers :**

For the kinds of diseases resulted from using street beverage 33.3% of the respondent mentioned typhoid,22.2% Shigellosis and 44.4 diarrhea and vomiting only .FAO and WHO.(1977) stated and alarmed that the danger expected calls a serious concern in areas where the standards of hygiene are low and increase of infections can not be avoided . The street vending foods should depend on hygienic conditions practices,

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procedures, and techniques which protect the community and prevent food-borne illness. It is a must that health authorities should make periodical inspection .Safety measures must be taken by health authorities such as observation of personal hygiene proper methods of washing and sanitizing food equipments, utensils , control of vermin and insects, potable water supply and sanitary waste disposals practices, El Zubeir, ( 2006).

According to the faecal contamination of different kinds of samples taken from studied area , it was found that 70% of the samples used for processing the cold beverages were contaminated with faecal coliform bacteria . The degree of contamination was different from one area to another. The highest degree of contamination was reached  $1.4 \times 10^6$  faecal coliform bacteria/ 100ml at El Souk El Arabi bus terminal while the lowest degree of contamination was 6.4 faecal coliform bacteria at Souk Elshouhada bus terminal .

All the ice samples used for the processing of cold beverage were contaminated with faecal coliform bacteria. Usually ice is processed from tap water, which is free from faecal bacteria, but the way of storage leads to the contamination. In most cases, ice was wrapped with sacks and put on the ground .The highest degree of contamination was  $4.3 \times 10^6$  faecal coliform bacteria /100 ml at Souk El Shabi Omdurman . The lowest degree of contamination was  $1.2 \times 10^6$  faecal coliform bacteria 100 ml at El Souk El Shabi Khartoum .

All samples of mango beverage (table,1)were faecally contaminated.

The highest degree of contamination was  $6.0 \times 10^6$  faecal coliform bacterial 100/ml at El Souk ElShabi bus terminal Omdurman .

All sample of guava (table ,2)beverage were found to be contaminated with faecal coliform bacteria .

The highest degree of contamination was  $8.1 \times 10^6$  faecal coliform bacteria / 100ml at El Souk El Shabi bus terminal Omdurman while the lowest degree was 6.4 faecal coliform bacteria. /100ml .

The highest degree of contamination was  $1.5 \times 10^7$  faecal coliform bacterial /100ml at Elshouhada bus terminal & the lowest degree of contamination . was  $1.2 \times 10^6$  faecal coliforms /100ml at El Souk El Arabi bus terminal .

The degree of contamination was found to be low in licensed places for purchasing cold beverage than unlicensed ones for guava and mango beverages and was the same for water and orange beverage.

The highest degree of contamination was found in guava beverage than other kinds of beverages .This may be due to the sensitivity of guava fruits to the spoilage and contamination . El Zubeir,,(2006).

The contamination of cold beverage is due to the untrained food handlers , bad disposal facilities , uncleanness of beverage of the area around and the shortage of public cycles .

### **Conclusion :**

The processing of cold beverages was made first by taking some fruits by hands and take the water by small bowl ,and here of course hands reach the water in the3 containers after they put all those beside sugar in the blender, then sieve the beverage in a big containers and put ice. These containers are left outside on the blenches ready to be served for all the people. In most cases these big containers are left uncovered and usually serve it using a small cup to fill the glasses . In this way also their hands reach the beverage. The ice for some purchasing places kept on the ground wrapped with sacks .Cold beverages are served in big quantities to large groups of people ,this way may provide opportunities to harm the health of the population due to the contamination coming from different sources include water, ice, fruits and the beverage handlers utensils used the area around.

### **Recommendation :**

There is a great need to protect the people from health hazards ,which may come from drinking street cold beverages,especially those beverages are served during the hot hours of the day ,where the people are very crowded to drink such beverages and no care is taken to cleaning facilities .



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## Appendices

Table(1):The Colony count, MPN Coliforms and Faecal Coliforms in Mango cold beverage(El Souk El Shabi , Khartoum )

Sample -pH	Colony Count\ml	MPN Coliforms\100ml	MPN Coliforms \ ml
1-----3.8	3.60E+05	2.4E+03	1.50E+02
2----- 3.7	4.60E+05	2.4E +02	2.30E +02
3----- 3.6	2.20E+06	2.4e +03	1.50E +03
4----- 4.4	3.10E+05	4.6E +02	2.60E +02
5----- 3.9	6.00E+04	4.1E +03	1.10E +03
6----- 5.6	5.00E+05	2.4E +03	1.10E +03
7----- 5.9	4.50E+05	3.4+ 03	1.50E +02
8----- 4.4	8.20E+04	4.5E +02	2.40E +02
9----- 4.8	4.60E+0.4	2.4E+03	2.00E +02
10----- 3.3	3.70E+05	2.4E+03	1.50E +03
11----- 4.0	3.80E+05	3.4E +02	2.30E +02

Table,(2) The Colony Count,MPN Coliforms and Faeceal Coliforms in Guava Cold Beverage (El Souk El Shabi ,Khartoum)

Sample no.	pH	Colony COUNT \ml	MPN Coliforms \100ml	MPNColiforms \100ml
1	4.4	6.40E +05	2.4E+02	1.50E+02
2	4.4	3.30E+05	1.1E+03	7.00E+00
3	4.6	4.50E+04	2.4E+03	2.00E+02
4	3.6	3.40E+05	3.1E+02	1.10E+02
5	4.0	7,70E +05	2.4E+03	1.10E+03
6	4.4	7.10 E +05	2.4E+03	1.50E+03
7	3.5	8.50E+04	2.3E+03	1.50E+03
8	3.8	2.60E+06	2.6E+03	2.10E+03
9	4.0	3.90E+05	2.4E+03	2.40E+02
10	4.0	2.30E+06	4.5E+02	1.60E+02
11	4.6	2.20E+06	4.6E+02	1.50E+02

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Orange(table 3) beverage samples 88% were found to be faecally contaminated

Table,(3):The Colony Count MPN Coliforms and Faecal Coliforms in Orange Cold Beverage .

Sample no.	pH	Colony Count\ml	MPN Coliforms\100ml	MPN Coliforms  100ml
1	2.6	2.25E+06	1.5E+03	1.50E+02
2	2.8	7.20E+04	2.1E+03	4.00E+00
3	3.4	4.50E+06	2.4E+03	2.00E+02
4	3.5	2.6E+05	2.4E+03	1.50E+02
5	4.6	2.20E+05	1.5E+01	7.00E+00
6	3.6	6.50E+05	1.1E+03	2.40E+01
7	3.8	2.50E+05	2.4E+03	1.50E+01
8	2.9	5.60E+04	2.4E+03	2.00E+02
9	4.0	2.50E+05	2.0E+00	<3
10	3.4	1.85E+05	2.4E+03	9.00E+00
11	3.9	2.20E+05	2.1E+03	6.00E+00