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Parasitological, Microbiological, Physiochemical, Mineral and Heavy Metal Concentration of Fresh Fruits and Vegetables Bought from Some Market

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Abstract
*Vegetables and fruits are highly nutritious, sources of vitamin, minerals, fibres etc and these are part of our daily diet. In recent time, there have been more production of vegetables and fruits as well as supply in local markets however, the main problem is associated with the quality of supplied vegetables and fruits due to heavy metals, microbiological contamination. Percentage availability of parasites on collected samples ranges from 20% to 80% while the total coliform count and plate count ranged from 2.1-71*10^2 and 4-56*10^2 respectively. Furthermore, the isolated parasites includes Ascaris lubricoides, Moniezia spp, Teania spp, Planaria, Unfertilized egg of ascaris among many others. The used samples also showed varying concentration of chloride, nitrate, sulphate and phosphate but with no traces of heavy metals. This showed that bought vegetables and fruits can be a vehicle for transmission of various diseases and intake of other chemicals into human body if not properly washed or preheated before ingestion.*

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I. Introduction

The dietary and economic values of consumable fruits and vegetables are exceptionally much recognized among the human populations. Vegetables are referred to as fresh edible portions of roots, stems, leaves or fruits of herbaceous plant. Many people from different origin consume vegetables raw or lightly cooked to preserve taste, nutrients and this practice of direct ingestion or slight cooking sometimes favour the transmission of food-borne parasitic and bacterial infections as well as heavy metals contamination. According to Damen et al., (2007), food normally becomes a potential source of human infection when contaminated during production, collection, transportation and preparation or during processing.

However, noteworthy predominance of infections coming about directly from consumption of parasitological sullied fruits and vegetables make it disturbing, troubling and a scientific cause of concern. Fruits and vegetables are known to play major part within the nutritional livelihood of Nigerians, especially in the country areas and cities where there's destitute socio-economic condition (Damen *et al*, 2007, Ogunleye *et al* 2010, Idahosa, 2011, Alade *et al*, 2013).

The rate of contamination and species of contaminant parasites varies based on climatic, ecological, and human factors. Therefore, local data about the contamination status and predisposing factors augments efforts for successful control of parasitic diseases.

According to Gelaw *et al.*, (2013), intestinal parasitic infections were described to be one of the most prevalent causes of disease in humans with an estimated figure of two billion individuals reported around the world to be infected with pathogenic and nonpathogenic intestinal parasites. In addition to the above, raw water and raw waste water sources used for irrigation are major sources of protozoan, bacteria and heavy metal contaminants and these bacteria and parasites are capable of causing food-borne diseases whose infections sometimes lead to serious health and socio-economic issues in some developed and many developing countries. According to the work of Scallan et al., (2011), it was reported that about 9.4 million food-borne illnesses are reported every year; of which, 200 thousand cases are caused by parasites.

Consumption of fresh vegetables and fruits regularly without proper washing has been linked or found to be associated with food-borne parasitic infections. Without much doubt, it is general conception that fruits and vegetables are vehicles that transmit parasites easily into individuals, especially when eaten raw or without peeling or washing (Hassan 2012).

Cryptosporidium, Cyclospora, Giardia, Entamoebahistolytica, Entamoeba coli, and Ascaris lumbricoides are considered to be the most common parasitic contaminants of fruits and vegetables (Heiser et al., 2003). Vegetables and fruits become contaminated with different parasitic stages by means of three fundamental pathways, via the contamination of raw vegetables and fruits on the farm during harvesting, through contaminated water used for irrigation or washing process, and through infected food handlers.

In developing countries, because of inadequate or even nonexistent systems for the routine diagnosis of food-borne pathogens, most disease outbreaks caused by contaminated vegetables go undetected, and the incidence of food-borne pathogens in food is underestimated. High rates of intestinal parasite diseases have been reported in communities that eat raw vegetables, which indicates that the consumption of raw vegetables is an important route of intestinal parasite transmission. Furthermore, consuming vegetables in a raw or slightly cooked form to protect or to preserve "heat-labile nutrients" may increase the risk of intestinal parasitic infections (Fawole and Oso 2004).

Vegetables are food substance meant to provide the body with nutrient and energy, however, there have been numerous health challenges attributed to the consumption of raw and semi-cooked vegetable that are directly ingested. Some vegetables and most fruits are eaten raw to retain the natural taste and preserve heat labile nutrients. However in doing so, these harmful microorganisms are ingested which can cause alongside unhygienic practice as well as poor human sanitation a major risk in the transmission of the parasitic and bacterial diseases as well as other chemical disruption of the body system.

The aim of this study is to conduct parasitological, Microbiological plate count, heavy metal, minerals and physiochemical investigation of selected fruits and vegetables sold in some selected markets in Ado Odo Ota Local Government.

II. Materials And Methods

Sample Collection

A total of hundred samples consisting of ten different fresh fruits and vegetable samples of apples, garden eggs, carrots, cherries, cabbages, mangoes, plums, golden melons, waterleaves, and tomatoes were gotten from different vendors within Igbesa and Lusada market of Ado Odo Ota Local Government Area.

Preparation of the sample

All the samples were prepared according to the method described by Adejayan and Olajumoke (2015) with slight modification of the process by placing the samples into sterile plastic plates containing 225ml to 1800ml of normal saline solution depending on the weight of the collected fruits to form a ratio of 1:10 and these were spun on the oscillator for several a periods of 15 minutes each for the removal of oval, cyst or larva. The residue was collected and centrifuged at 1000rpm for 10 minutes after which the suspensions were collected and used for analysis.

All the glass wares used in this study were sterilized in a hot air oven at 160 ˚C for 2 hrs. The other materials were sterilized by autoclaving at 121 ˚C for 15 minutes.

Parasitological Examination (Microscopy)

Two drop of each of the oscillated water residues were placed on a clean slide, followed by the use of a cover slip were observed the x10 and x40 objective lens of the microscope for observation of different parasites and their eggs.

Microbiological Plate Count

Nutrient agar (NA) and Eosine Methylene Blue agar (EMB) were prepared following manufacturer's procedure and were allowed to cool to bearable temperature before used for plating. 0.2ml of the various oscillated water were inoculated using pour plate method and were incubated for 24hours for both EMB and NA respectively.

Heavy metals and Minerals Analysis

Sample dissolution for metal analysis (Wet oxidation of samples) for each water residue was done using the following reagents:

i. Conc. Nitric acid (HNO_3)AR
ii. Hydrochloric acid (HCl) AR
iii. Digestion system
iv. Digestion flasks

A known volume (10ml) of homogenous sample was taken into the digestion flask. 10ml volume of nitric per chloric acid was added to the sample and digested until clear solution or dense white fume is attained signaling the end of the digestion The solution was washed into 50ml volumetric flask and filtered (SSSA, 1996)

Analysis on AAS

The resultant digest was then determined on an Atomic Absorption Spectrophotometer (AAS) at the specific wavelength of each metal. Metals were analyzed using Model 210 VGP of the Buck Scientific AAS series with air-acetylene gas mixture as oxidant while Na and K were determined using Corning 410 model Flame photometer. Solutions from above digestion were aspirated after calibrating the equipment for each element. The results were recorded as mg l^{-1} of solution and were calculated to mgL^{-1} of sample using the aliquot of sample taken as a denominator of the final digest volume (25 ml).

<u>Calculation</u>
mg l^{-1} sample = digest conc. x dilution factor
digest conc = Analyte reading on AAS/Flame photometer
DF = <u>Vol of digest</u>
Initial vol. of sample
vol of digest = Final volume of digested or extracted sample

Physiochemical Analysis
Sample dissolution for physiochemical analysis (using powdered reagent) for each water residue and this was done using the following reagents of Sulphate, Nitrate, Iron, Chloride, Phosphate, Calcium, Magnesium, and Fluoride.
The spectrophotometer machine was turned on and let to sit for 15mins before running the samples .The cuvette is cleaned up and when handling, avoid touching the sides which the light will pass through. 10ml of the sample is poured in a cuvette along with 2g each of the reagent mentioned above and shaken thoroughly to mix, the sides of the cuvette is cleaned before placing it into the spectrophotometer to avoid interference from dirt or dust particles. The cuvette is placed in the spectrophotometer and the timer is set for 3 minutes to obtain accurate result. These analysis are repeated for all other reagent.

III. Results

The results obtained are as represented in the tables below

Table 1: Percentage Availability of Parasites on Collected Samples

No	Samples	Total Number of Samples Collected	Percentage of Sample positive for Parasite	Percentage of Sample Negative for Parasite
1	Cherry	10	60% (6)	40% (4)
2	Garden Egg	10	50% (5)	50% (5)
3	Plum	10	20% (2)	80% (8)
4	Water leaf	10	70% (7)	30% (3)
5	Tomatoes	10	80% (8)	20% (2)
6	Cabbage	10	60% (6)	40% (4)
7	Golden Melon	10	30% (3)	70% (7)
8	Mangoes	10	50% (5)	50% (5)
9	Carrot	10	40% (4)	60% (6)
10	Apple	10	30% (3)	70% (7)
	Total/Average	100	49%	51%

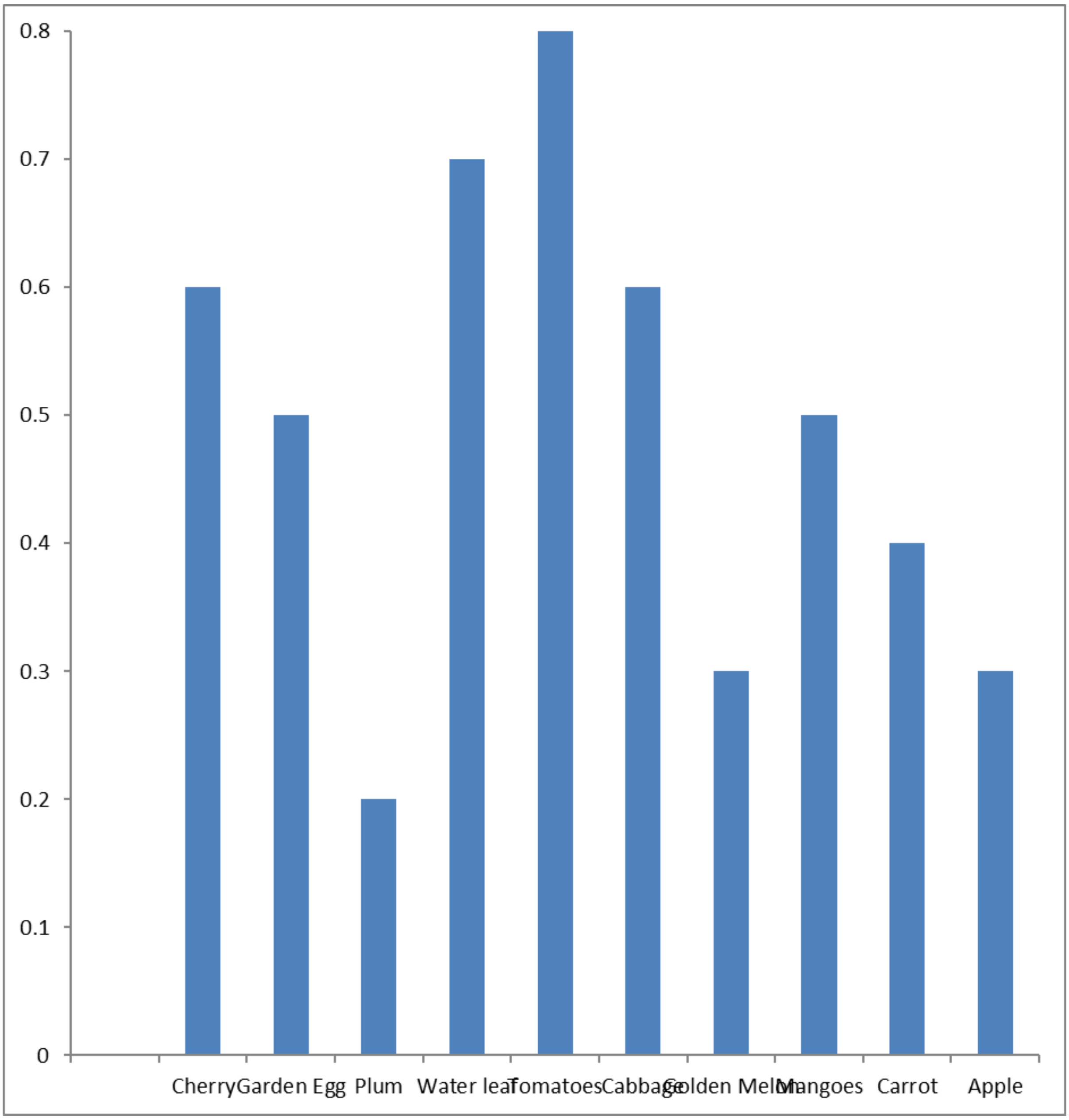

Figure 1: Percentage Availability of Parasites on Collected Samples

Table 2: Average Parasitological Evaluation of Collected Fruits and Vegetable Samples

Sample	Microorganisms founds During Microscopy
Cherry	*Ascaris Lubricodes, Moniezia spp, Diphylidium Caninum, unfertilized egg of Ascaris*
Garden Egg	*Teania Spp, Planaria, unfertilized egg of Ascaris, Moniezia spp, Advanced larvae of Enterobium Vermicularis*
Plum	*Teania Spp, Toxacaris Leonina, Moniezia spp*
Water Leaf	*Toxaseans Leonina, Teania Spp, Ascaris male, Ascaris female.*
Tomatoes	*Moniezia spp, Pseudomonas Caerum, Septona Lycopesium*
Cabbage	*Teania Spp, unfertilized egg of Ascaris, Hymenolepsis nana*
Golden Melon	*Ascaris male, Strongyloides Stecoralis, Schistosomas japonicum*
Mangoes	*Hook worm, Fruit fly maggot (Anastrepha spp.), Aulacaspis Turorcularis.*
carrot	*Entamoeba coli, Ascaris lumbricoidis, Trichuris trichura*
Apple	*Moniezia spp, Schistosomia mansuni, unfertilized egg of Ascaris*

Table 3: Average Coliform and Total Plate Count

Sample	Microbial Count After 24 hours (x10^{2}cfu/mL)	
	EMB	NA
Apple	6	4
Garden Egg	3	40
Cabbage	20	56
Tomatoe	71	21.5
Carrot	21.2	39.6
Waterleaf	20.4	37.3
Golden Melon	2.1	5.7

Table 4.4: Heavy metal, Physiochemical, and Mineral Analysis

sample	AP	CAB	CaR	CH	GE	GM	MN	PL	TO	WL	MV
Parameters	Mg/1	Mg/1	Mg/1	Mg/1	Mg/1	Mg/1	Mg/1	Mg/1	Mg/1	Mg/1	Mg/1
Sodium	1.760	11.880	1.320	4.840	2.220	0.880	1.770	1.320	3.960	3.080	0.440
Potassium	0.000	35.770	2.940	11.270	0.000	0.980	4.910	0.000	15.190	5.390	0.000
Chloride	0.143	0.093	0.101	0.000	0.000	0.059	0.065	0.000	0.083	0.000	0.000
Nitrate	0.068	0.081	0.075	0.085	0.053	0.075	0.056	0.077	0.061	0.058	0.037
Sulphate	0.453	0.379	0.303	0.355	0.267	0.338	0.289	0.364	0.399	0.321	0.269
Calcium	1.810	5.220	2.590	4.290	2.040	3.120	3.360	2.870	2.960	3.230	0.410
Magnesium	0.047	3.185	0.314	0.671	0.049	0.191	0.354	0.052	0.232	0.928	0.003
Copper	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Zinc	0.039	0.029	0.087	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Manganese	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Iron	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cadmium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Chromium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cobalt	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nickel	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lead	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Phosphate	0.215	2.153	0.538	1.937	1.076	0.215	1.184	0.000	0.323	0.000	1.292

IV. Discussion

Parasitological Test

A total of 10 fruit and vegetable samples were analysed. From the analysis, a host of micro organisms were found, which includes Ascaris lubricodes, Moniezia spp, Teania spp, Toxacaris leonina, Hook worm, Fruit fly maggot etc, Parasites like Trichuris trichura, Hymenolepis nana, Diphilisium caninum , Enterobium vermicularis, Entermoeba coli, Ascaris lumbricoides, Taenia spp, planaria caused symptoms like abdominal discomfort, diarrhea, loss of appetite, anemia, stunted growth, fever, nausea e.t.c .According to the Parasitological analysis results the percentage availability of parasites on collected samples range from 20% to 80%. The most prevalent micro organisms found was Moniezia spp, Teania Spp and unfertilized egg of Ascaris. These micro organisms can be very harmful to both humans and animals, causing cramped vomiting, seizure (Cysticercosis) amongst others. This is in accordance with a study carried out by (Adejayan and Olajumoke 2015), which found high levels of intestinal parasite like Ascaris lubricodes, Hook worm, Strongyloides stercoralis, as well as high number of eggs or larvea in vegetables like cabbage, carrot, and lettuce.

Total Plate Count

After the microbial culture of the samples for 24 hours, the total colony count and plate count range from 2.1-71*10^2 cfu/ml and 4 -56*10^2cfu/ml respectively the sample with the highest colony count on EMB agar was Carrot (21.2 x10^2 cfu/ml), while the highest colony count on Nutrient agar was Cabbage(56.0x10^2 cfu/ml). The sample with the lowest colony count on EMB and Nutrient agar was Apple, 6x10^2 cfu/ml and 40x10^2 cfu/ml respectively. Which is in accordance to a study done by (Angela, 2010) where all the fruits and vegetables sampled in this study were contaminated. With apples having the lowest microbial load (9 × 10 5 cfu/ml) of all the fruits and vegetables sampled, while spring onions and pineapple had the highest microbial load (3.0 × 10 7 cfu/ml).

Heavy metal

The heavy metals found in the 10 samples of fruits and vegetables includes potassium, zinc, copper, iron, cobalt, nickel .There is a trace of zinc in apple (0.039), cabbage(0.029) and carrot (0.087) sample, and also the presence of iron in carrot(0.047). Other elements of heavy metal analysis shows no (0) concentration, except for zinc, iron and phosphate. It is seen that cabbage has the highest concentration of heavy metal which is (35.770) in samples.

From the investigation carried out in the determination of the concentration of heavy metals in fruits and vegetables obtained from three selected markets in Anambra State, by (Chinazo, 2020) it was observed that some of the fruits and vegetables showed low levels of heavy metals while some showed high levels of heavy metals in them. When compared with the standard permissible limit set by the FAO/WHO (Food and Agricultural Organization and World Health Organization), the levels of heavy metals were observed to be higher than the safe limit set by the FAO/WHO. This may be from the high level of pollution in the area. .

4.2.4 Mineral analysis

In the result of the mineral analysis carried out copper, zinc, chromium, calcium, potassium, chloride, manganese and magnesium.In the mineral analysis, it is observed that sodium, potassium, chloride, magnesium, and calcium, are all present within the permissible limit. In the fruit and vegetable sample, there is low concentration of chloride and cumulatively high concentration of potassium in the sample.

The research work done by (Usunobun and Egharebva, 2014) contributes to the understanding of mineral concentration of fruits and vegetables. The Leafs of vegetables has somewhat higher levels of mineral than fruits. The values were more or less comparable with the data reported by different researchers in different country. The leaves are good source of Fe, Cu, K, and Mn, which meet the recommended daily allowance. Higher Potassium content further confirmed that the leaves of this plant could serve as better diets for hypertensive. High concentration of Ca indicates that the pumpkin diet could be recommended for a person with tooth and bone problems.

Physiochemical analysis

In the physiochemical analysis sulphate, phosphate, chloride, and nitrate were analysed. During physiochemical analysis, it was observed that there are varying concentrations of physiochemical materials such as phosphate, nitrate, chloride and sulphate present in the samples

In conclusion, this study highlighted the risk of consuming unwashed fruits and vegetables and their potential source as a means of transmission for intestinal parasites and pathogenic bacteria that are associated with soil to humans as well as sources of chloride, sulphate, nitrate and phosphate. The fruits and vegetables contamination with the pathogenic parasites, bacteria and different chemical compounds poses health risks to the consumers if consumed without proper cleaning and or cooking.

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