Microbial and Heavy Metals Contaminations among the Commonly Sold Vegetables in Lagos State, Nigeria

Odumosu, B.T., Njoku, E.J., Fatunsin, O.T. Adeogun, O.O. and Alabi, O.S.
[1]Department of Microbiology, Faculty of Science University of Lagos, Akoka-Yaba, Lagos
[2]Department of Chemistry, Faculty of Science, University of Lagos, Akoka-Yaba, Lagos
[3]Department of Botany, Faculty of Science, University of Lagos, Akoka-Yaba, Lagos
[4]Department of Pharmaceutical Microbiology, Faculty of Pharmacy University of Ibadan, Ibadan
*Corresponding Author: deliniz@yahoo.com; bodumosu@unilag.edu.ng
+2348034515048

Abstract: Assessments of heavy metals and microbial contaminations of vegetables cannot be overemphasized as vegetables are important components of human diet. Intake of heavy metal-contaminated vegetables poses high risk to human health. Heavy metal concentrations and microbial contamination in twelve (12) most consumed vegetables *Talinum triangulare*, *Telfairia occidentalis*, *Vernomia amygdalina*, *Amaranthus hybridus*, *Ocimum gratissimum*, *Celosia argentea*, *Taraxacum officinale*, *Piper guineense*, *Lactuna sativa*, *Gnetum africanum*, *Gongronema latifolium*, and *Pterocarpus mildraedii* was carried out using standard laboratory procedures. Antibiotic susceptibility testing against 6 structurally unrelated antibiotics (clindamycin, cefoxitin, cloxacillin, gentamycin, erythromycin and ciprofloxacin) were done on the isolates obtained using disk diffusion method. Total of 36 bacteria isolates were obtained comprising of *Staphylococcus aureus* (12, 33.3%), *Bacillus* spp. (23, 63.9%), *Micrococcus* spp. (1, 2.80%) which were identified based on cultural and biochemical identification. Twenty three Fungal isolates involving *Aspergillus acelatus* (7, 30.43%), *A. fumigatus* (7, 30.43%), *A. niger* (5, 21.74%) and *Penicillium* Spp (4, 17.40%) were identified based on cultural and fungal staining technique. The overall number of susceptibility for ciprofloxacin (72%), erythromycin (61%) and gentamicin (50%) were recorded. Antibiotic resistance were recorded for clindamycin (66%), cloxacillin (61%) and cefoxitin (45%). The heavy metals concentration on the examined vegetables were above WHO acceptable limit of 0.3mg/kg, 0.2mg/kg and 1.30mg/kg for Pb, Cd and Cr respectively. The bacteria isolated harbored antibiotic resistance mechanisms against antibiotics which calls for urgent measures to minimize all possible routes for contaminations

Keywords: Heavy metals, bacteria and fungal contamination, vegetables

INTRODUCTION

Environmental pollution is a major challenge in developing nations. Anthropogenic activities such as domestic waste and farm run offs contributes largely to pollutions of the aquatic environment (Munaza et al., 2016). Anthropogenic activities and environmental pollutions are two major sources of heavy metals contamination in the soil (Zwolak *et al*., 2019). Aggregation of heavy metals in the soil which are often passed down to the food chain poised severe public health risks (Salawu *et al*., 2015; Musa *et al*., 2017). Metals such as cadmium (Cd), chromium (Cr), and lead (Pb) are characterized by density, atomic number/weight, chemical properties and toxicity (Heidarieh *et al*., 2013). Such metals accumulate in the tissues and organs of human and animals. When a large concentrations of heavy metals are ingested, it causes stunted growth, weight loss and severe damage to vital organs of plants and animals bodies (Singh *et al*., 2010; Blowes, 2002). Lead pollution in an environment accumulate in the commonly consumed vegetables through air, water and food contaminations (Apte *et al*., 2019). Pollutants in irrigation water, farm soil or pollution from the highways traffic may be attributed to the elevated amounts of Pb in some plants (Balali *et al*., 2020).

Fruits and vegetables are considered an essential component of a healthy diet and are usually consumed in Nigeria (authors personal observation). They are low fats, energy-dense, abundant in vitamins, minerals and other bioactive compounds, as well as being an effective source of fiber (Rekhy and McConchie, 2014). Vegetables are either consumed raw, cooked and/or are used as ingredients in a wide spectrum of foods and can be grouped into: green leafy vegetables, bulbs, pulses and legumes, roots and tubers, vegetable fruits (Sue and Demand, 2015). A high intake of fruits and vegetables in the diet is positively associated with the prevention of cardiovascular disease, cancer, diabetes and osteoporosis (Callejon *et al*., 2015).

Despite the beneficial aspects of fruits and vegetables, epidemiological investigations ranked raw fruits and vegetables as the second most common source of foodborne illness (Wang *et al*., 2014; Bhunia, 2018). Contamination of vegetables by *Salmonella* spp, *Shigella* spp., *Escherichia coli* O157:H7, *Campylobacter spp* and *Listeria monocytogenes* was previously reported (Ayhan *et al*., 2011). Over the years, foodborne outbreaks associated with vegetables that are incompletely processed or consumed raw have increased due to conducive nature of the vegetables that supports and encourage the growth and survival of many pathogens (Sarker *et al*., 2018).

Furthermore, the impact of antibiotics uses, directly or indirectly during farm practices, is also a serious concern (Guetiya-Wadoum et al., 2016). Although this subject has been emphasized especially in food producing animals (Odumosu *et al*., 2016), less attention is being paid to its clinical impact on vegetables. Improved agricultural mechanization and modern farm practice benefits when inappropriately using antibiotics may develop resistance to these sub-lethal concentrations and disseminate it to other pathogenic strains.

Recently, efforts have been devoted to prolonging the shelf life of vegetables, however, there is a need to determine the antibiotic susceptibility patterns of the bacteria species associated with vegetables due to constant changes caused by anthropogenic activities. This study aimed to investigate the level of heavy metals and microbial contamination level among commonly consumed vegetables obtained from various markets in Lagos state, Nigeria as well as to determine the antibiotic susceptibility pattern of the isolated microorganisms.

MATERIALS AND METHODS

Sample Site

The sampling sites were located in various markets across Lagos metropolis and they include: Bariga, Gbagada, Ikeja, Oyingbo, Shomolu and Yaba. Samples were obtained from traders at the point of sales in those places mentioned above (Figure 1).

Figure 1: Map of Lagos state depicting the sample collection sites in red boundaries.(Infostride, 2019).

Sample collection

The vegetable samples in this study were purchased for a period of two months (May and June, 2019) with four days interval in a polythene bag from the market and transported immediately to Department of Microbiology, University of Lagos laboratory. The following vegetable samples were purchased for the purpose of this study: Waterleaf (*Talinum triangulare*), Fluted pumpkin leaf (*Telfairia occidentalis*), Bitterleaf (*Vernomia amygdalina*), African spinach (*Amaranthus hybridus*), African basil (*Ocimum gratissimum*), Lagos spinach (*Celosia argentea*), Dandelion greens (*Taraxacum officinale*), Ashanti pepper (*Piper guineense*), Lettuce (*Lactuna sativa*), African Jointfir (*Gnetum africanum*), Bush buck plant (*Gongronema latifolium*), Padouk (*Pterocarpus mildraedii*).

Isolation of Bacterial and Fungal Isolates

Isolation of bacteria and fungi were carried out according to the protocols described by Sarker *et al*. (2018). Briefly, 5g of each vegetable sample was thoroughly washed with sterile distilled water, weighed and allowed to dry in air and then pulverized. Serial dilutions of the powdered vegetables were carried out with a sterile distilled water. From the final dilution of 10^{-5}, 0.1 ml was inoculated separately on the prepared nutrient agar (NA), MacConkey agar (MCA), deoxycholate citrate agar (DCA) and potato dextroxe agar (PDA) using spread plate technique. The seededplates were incubated at 37^{o}C for 24 h for the bacteria isolation while 28^{o}C for 5 days was used for the fungal isolation. The plates were observed for colonies development. Mixed Bacteria colonies were sub-cultured to obtain pure isolates which were gram stained and viewed under the microscope. Standard biochemical tests for the characterization of bacteria were carried out.

Mixed Fungal isolates were also sub-cultured to obtain pure isloates before being identified using cultural and morphological features. Colonies were stained using lactophenol and viewed under the light microscope with x10 and x40 objective lenses (Oyeleke and Manga, 2008).

Heavy Metals Analyses

Presence of heavy metals were analyzed using the method adopted from Taghipour and Mosaferi, (2013).

Briefly, 20g of each vegetable samples was weighed, washed, air dried and then transferred into hot-air oven at a temperature of 80°C for 8 h and the dried samples were crunched to small sizes. Samples were digested with Aqua regia (nitric acid: Hydrochloric acid 3:1) and the concentration of potentially toxic metals (PTMs) were determined using a calibrated Buck Scientific Model 210 VGP, Serial Number 1619 Flame Atomic Absorption Spectrophotometer (AAS) as in EPA The following heavy metals were determined; lead (Pb), cadmium (Cd) and chromium (Cr)

Antibiotic susceptibility testing

Susceptibility of the test bacteria to commonly used antibiotics was carried out by disk diffusion method on Mueller Hinton (HiMedia, India) agar plates as previously described with slight modifications (Bauer *et al*., 1996). The 24 hrs McFarland standard culture of each isolate was swabbed on the agar plates surface using sterile swab sticks. The 6 mm antibiotic was firmly pressed on the surfaces using sterile calipers. The following antibiotics disk were used in this study: clindamycin (CLI 2µg), cefoxitin (FOX 30µg), cloxacillin (OB 5µg), gentamicin (GEN 30µg), erythromycin (ERY 15µg) and ciprofloxacin (CIP 5µg) (Abitek USA). The agar plates were incubated at 37^{o} C for 24 hrs. after incubation the diameter of zone of inhibition for each antibiotic disc was measured in millimeters and interpreted according to the Clinical Laboratory Standards Institute (CLSI, 2019).

RESULTS

Total of 36 bacteria isolates were obtained after analyses of the 12 types of vegetables collected across Lagos. Preliminary identification and standard biochemical tests revealed the presence of three bacteria genera: *Staphylococcus*, *Bacillu*s and *Micrococcus*. Predominant species from these genera were *Bacillus* species (23, 38.98%), *S. aureus* (12, 20.34%) and *Micrococcus leteus* (1, 1.70%)(Table 1). Total of 21 fungal isolates were characterized from analyses. The results of the cultural and morphologies revealed the presence of *Aspergillus acelatus, A. fumigatus, A. niger* and *Penicillium species* and yeasts (Table 1).

All the isolated bacteria and fungi were distributed in varying proportions among the selected vegetables. *Bacillus* spp was highly prevalent among all the vegetables followed by *S. aureus* while *Aspergillus* spp was more prevalent in all the vegetables while *Penicillum* spp was the least prevalent. The antibiotic susceptible testing of the bacteria isolates are represented in Table 2. Overall highest number of susceptibility was recorded for ciprofloxacin (72%), erythromycin (61%) and gentamicin (50%) while overall number of resistance was recorded for clindamycin (66%), cloxacillin (61%) and cefoxitin (45%). Bacillus spp. resistance to clindamycin, cloxacillin, and erythromycin was 17 (73.9%), 15 (65.2%) and 10 (43.8%) respectively while *Staphylococcus aureus resistance* showed 6 (50%) resistance to cefoxitin, clindamycin and cloxacillin (Table 2).

The samples were analyzed for heavy metals such as Cd, Cr and Pb . The Cd, Cr and Pb in this study ranged between 0.00 and 11.5 ppm, 0.00 and 21.00 ppm, 0.10 and 16.50 ppm respectively (Table 3). Cd concentration was highest in Bitter leaf (11.5mg/kg) and Bush buck (8.00mg/kg), while highest concentration of Cr was found in Ashante pepper (21.00mg/kg) followed by Lagos spinach and waterleaf having 10mg/kg each. Pb concentration was found in highest concentration in scent leaf and Dandelion greens with 16.50mg/kg and 14.00mg/kg respectively.

Table 1. Frequency of microorganisms isolated from 7 vegetables samples

Microorganism	No of isolates	Percentage
Bacilius spp	23	38.98
Staphylococcus aureus	12	20.34
Micrococcus spp	1	1.70
Aspergillus fumigates	7	11.86
Aspergillus acelatus	7	11.86
Aspergillus niger	5	8.48
Penicillium spp	4	6.78

Table 2. Antibiotic susceptibility testing of bacteria isolated from the vegetables

Antibiotics	***Bacillus* spp**			***Staphylococcus* spp**			***Micrococcus* spp**		
& *Pattern*	*R*	*I*	*S*	*R*	*I*	*S*	*R*	*I*	*S*
Cefoxitin	9 (25.0%)	5 (13.89%)	9 (25.00%)	6 (16.67%)	2 (5.55%)	4 (11.11%)	-	-	1 (2.78%)
Ciprofloxacin	3 (8.33%)	6 (16.67%)	14 (38.89%)	1 (2.78%)	-	11 (30.55%)	-	-	1 (2.78%)
Clindamycin	17 (47.22%)	2 (5.55%)	4 (11.11%)	6 (16.67%)	4 (11.11%)	2 (5.55%)	-	-	1 (2.78%)
Cloxacillin	15 (41.67%)	4 (11.11%)	3 (8.33%)	6 (16.67%)	4 (11.11%)	3 (8.33%)	-	-	1 (2.78%)
Erythromycin	10 (27.78%)	10 (27.78%)	3 (8.33%)	4 (11.11%)	4 (11.11%)	4 (11.11%)	-	-	1 (2.78%)
Gentamicin	3 (8.33%)	9 (25.00%)	11 (30.55%)	1 (2.78%)	5 (13.89%)	6 (16.67%)	-	-	1 (2.78%)

Key: R = resistance; I= intermediate; S=sensitive

Table 3: Result of heavy metal analysis in commonly consumed vegetables

Cd (N = 3)		Cr (N = 3)		Pb (N = 3)	
Mean Conc. (mg/kg)	SD (±)	Mean Conc. (mg/kg)	SD(±)	Mean Conc. (mg/kg)	SD(±)
5.5	0.20	0.0	0.05	0.1	0.05
2.5	0.20	21.0	0.10	4.5	0.10
11.5	0.50	8.0	0.10	8.0	0.10
8.0	0.10	6.0	0.10	1.5	0.10
3.5	0.20	3.0	0.20	14.0	0.20
1.0	0.20	7.5	0.20	2.0	0.20
0.0	0.06	5.5	0.10	7.0	0.10
1.0	0.06	10.5	0.25	5.0	0.25
0.0	0.06	6.0	0.20	0.5	0.20
1.0	0.01	3.5	0.20	16.5	0.20
2.5	0.10	10.0	0.20	4.0	0.20

*Conc. of Plant mg/kg WHO acceptable limits: Cd: 0.2 mg/kg; Pb: 0.3 mg/kg; Cr: 1.30 mg/kg;

DISCUSSION

Microbial contamination of food is a serious concern in ensuring food safety and prevention of non-communicable diseases associated with pathogens harbouring the various food substances. The present study examined commonly consumed vegetables in Nigeria for the presence of microbial contamination and heavy metal to ascertain the level of public health risk associated with these vegetables. The overall findings of the present study showed that the investigated raw vegetables contained high microbial contamination with the exemption of coliform bacteria and heavy metals at unacceptable limits.

The selected vegetables were found to harbored both fungi and bacteria isolates at varying populations (Table 1). All the vegetables harboured both *Bacillus* spp. and *Staphylococcus aureus* while *Micrococcus* spp. was found in only *O. gratissimum*. The microbial contamination level for the vegetables were high and it comprises majorly of 3 genera within the context of our characterization methods. Interestingly, no coliform bacteria was isolated from any of the tested vegetables thus suggesting there were no fecal contaminations on the samples. In the current study, *Staphylococcus aureus*, *Bacillus* spp and *Micrococcus* spp. were the bacteria representatives isolated while *Aspergillus* spp. (*A. acelatus, A. fumigatus, A. niger*), *Penicillium* **s**pp. and yeasts were the fungal isolates.

The antimicrobial susceptible test results revealed a disturbing high resistance to antibiotics especially to erythromycin (43%), cloxacillin (65%) and clindamycin (73%) by *Bacillus* spp. In comparison to *S. aureus* with lower resistance profile and *Micrococcus* spp. with the highest susceptibility to all the antibiotics investigated. Although incidence of foodborne outbreak with *Bacillus* and *Staphylococcus* could be associated with external contamination as previously (Ayhan *et al*., 2011). Among the antibiotics tested, there were high susceptibilities to ciprofloxacin and gentamicin by all the tested bacteria affirming the potency of the two broad-spectrum antibiotics.

Heavy metal contaminated water and food has been consistently recognized as one of the major sources of increase human blood lead level as well as cadmium contents in foods (Satarug *et al*. 2010; Brown and Margolis, 2012). They also pose high risk to health and can cause several issues if continued unabatedly.

Heavy metal contaminated water and food has been consistently recognized as one of the major sources of increase human blood lead level as well as cadmium contents in foods (Satarug *et al*. 2010; Brown and Margolis, 2012). They also pose high risk to health and can cause several issues if continued unabatedly. In the current study, the overall concentration of the heavy metals has been described (Table 3). The concentration of lead in the vegetables ranged from 0.10 to 16.50 mg/kg, cadmium range from 1.00 to 16.5 mg/kg while chromium content was in the range of 3.00 to 21.00mg/kg. The lead contents of the vegetables in this study is above the FAO/WHO safe limit of 0.3 mg/kg (JECFA, 2018) and it is also higher than a previous study in Nigeria which reported a range of 4.27 to 12.04 mg/kg. The observed concentration of lead in these leafy vegetables significantly pose a threat to public health food consumption. Although previous studies have indicated that traces of Pb at approximately 29ng/g diet is important for the body especially during cell development and reproduction (Assi *et al*., 2016). However increased Pb concentration in adult's blood may affect certain functions of the body such as cognitive performance, delayed puberty, central nervous system, kidney and fertility problems etc.

Food and water remains one of the main source of cadmium in the body where it accumulates principally in the liver and kidneys. Cadmium is a non-essential mineral in the body, it is used mainly as an anticorrosive agent on steel and as pigments in plastics (Friberg *et al*., 1986; Divrikli *et al*., 2006). The cadmium contents of the vegetables investigated in the present study were also higher than the FAO/WHO maximum acceptable limits for cadmium which is 0.02 mg/kg. Compared to previous studies that reported low concentration of 0.049 mg/kg (Muhammad *et al*., 2008) and 0.09 mg/kg (Sobukola *et al*., 2010), the range of cadmium content of the vegetables in the present study was found to be higher and it is worrisome. The vegetable samples were also found to be contaminated with chromium, although some of the investigated vegetables were without chromium contents. The range of chromium content of vegetables in the present study was from 0.00 to 21.00 mg/kg. It was also observed that the concentrations of the chromium metal on the washed vegetables were above tolerable limit of maximum value 1.30ppm of chromium in vegetables (WHO, 2000). while the result of this study showed that 71% of the samples analyzed were above 1.30 ppm; however, it is important to note the need for continuous monitoring of the metal in the commonly consumed vegetables.

CONCLUSION

The present study reveals accumulation of heavy metals and microbial contamination in commonly consumed vegetables in Lagos state, Nigeria. The concentrations of the heavy metals were all above WHO acceptable limit which suggests ingestion of potentially toxic vegetables while the nature of the isolated bacteria and fungi are potentially dangerous for human consumption. It is therefore recommended that the source of these metal accumulations and contaminationsbe properly traced and identified to ensure efficient solutions are provided in order to prevent possible food disease outbreak or poisoning associated with the consumption of food contaminated with dangerous microorganisms and high level heavy metal contents.

REFERENCE

Apte, A., Bradford, K., Dente, C., & Smith, R. N. (2019). Lead toxicity from retained bullet fragments: A systematic review and meta-analysis. *Journal of Trauma and Acute Care Surgery*, *87*(3), 707-716.

Assi, M.A., Hezmee, M.N.M., Haron, A.W., Sabri, M.Y., Rajion, M.A. (2016). The detrimental effects of lead on human and animal health. Veterinary World.9:660–671. doi: 10.14202/vetworld.2016.660-671.

Ayhan, T., Ufuk, B., and Sine, O. T. (2011). Different Decontamination Treatment on Microbial Population of Leafy Vegetables. *Journal of Geographic Information and Decision Analysis.* 36(1): 9-13.

Balali, G. I., Yar, D. D., Afua Dela, V. G., & Adjei-Kusi, P. (2020). Microbial Contamination, an Increasing Threat to the Consumption of Fresh Fruits and Vegetables in Today's World. *International Journal of Microbiology*, *2020* :1 – 13

Bauer, A. W., Kirby, W. M. M., Sherris, J.C., Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology. Vol 45, Issue 4_ts, 493-496.

Bhunia, A. K. (2018). *Foodborne microbial pathogens: mechanisms and pathogenesis*. Springer. Pg 1 – 23

Blowes, D. (2002). Tracking hexavalent chromium in ground water. Science. 295:2024- 2025.

Brown M. J., Margolis, S. (2012). Lead in drinking water and human blood lead levels in the United States. MMWR Morbidity and Mortality Weekly Report.61:1-9.

Callejon, R.M., Rodríguez-Naranjo, M.I., Ubeda, C., Hornedo-Ortega, R., Garcia- Parrilla, M.C., Troncoso, A. M. (2015). Reported foodborne outbreaks due to fresh produce in the United States and European Union: Trends and causes of foodborne Pathological Diseases. 12: 32 – 38.

Clinical and Laboratory Standards Institute (2019).*Performance standards for antimicrobial susceptibility testing;* 29th ed, CLSI supplement M100 2162- 2914. Wayne, PA

Divrikli, U., Horzum, N., Soylak, M., Elci, L. (2006). Trace heavy metal contents of some spices and herbal plants from western Anatolia,Turkey. *International Journal of Food Science and Technology*. 41: 712-716.

Friberg, L., Kjellstrom, T., Nordberg, G. F. (1986). Friberg, L., Nordberg, G. F., Vouk, V. (eds.) Handbook on the toxicology of metals, 2nd edition, Elsevier Science Publishers, Amsterdam, New York.

Guetiya-Wadoum, R.E.; Zambou, N.F.; Anyangwe, F.F.; Njimou, J.R.; Coman, M.M.; Verdenelli, M.C.; Cecchini, C.; Silvi, S.; Orpianesi, C.; Cresci, A.; et al. (2016). Abusive use of antibiotics in poultry farming in Cameroon and the public health implications. Br. Poult. Sci. 57, 483–493.

Heidarieh, M., Maragheh, M. G., Shamami, M. A., Behgar, M., Ziaei, F. (2013). Evaluation of heavy metal concentration in shrimp (*Penaeussemisulcatus*) and crab (*Portunuspelagicus*) with INAA method. Springer Plus. 2:72 – 75.

Infostride 2019. https://www.theinfostride.com/pssdc-collaborate-with-lasu-for-certificate-course-in-local-government-administration/ (Date accessed 5th March 2020)

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (2018). Joint FAO/WHO food standards programme codex committee on contaminants in foods.CF/12 INF/1 March 2018.

Munazah, N., Uzma, T., Shaista, A. (2016). Environmental pollution and sustainable development in developing countries. Pak Dev. Revs 55(4) 589-604

Muhammad, F., Farooq, A., Umer, R. (2008). Appraisal of heavy metal contents in different Vegetables grown in the vicinity of an industrial area. *Pakistan Journal of Botany*. 40(5): 2099-2106

Musa, Y., Nita, D., Emmanuel, A., Galo, Y. S., Louis, H., Innocent, J. (2017). Determination of the level of heavy metals in liver and kidney of cow and goat used as meat source in Mubi Adamawa State, Nigeria. *International Journal of Research in Environmental Science*. 3(3):1-8.

Odumosu B.T., Olabayo A., Hannah D.A., Idowu O. (2016). Antibiotic suscpetiblity pattern and analysis of palmsid profiles of Pseudomonas aeruginosa from human, animal and plant. SpringerPlus 5:1-7

Oyeleke, S.B., Manga, S.B. (2008). Essentials of laboratory practical in microbiology. 20-33.

Rekhy, R., McConchie, R., (2014). Promoting consumption of fruit and vegetables for better health. Have campaigns delivered on the goals? Appetite. Aug;79:113–23. doi: 10.1016/j.appet.2014.04.012.

Salawu, K., Barau, M. M., Mohammed, D., Mikailu, D. A., Abdullahi, B. H., Uroko, R. I. (2015) Determination of some selected heavy metals in spinach and irrigated water from Samaru area within Gusau metropolis in Zamfara State, Nigeria. *Journal of Toxicology and Environmental Health Sciences*. 7(8):76-80.

Sarker, M. A. R., Haque, M. M., Rifa, R. A., Ema, F. A., Islam, M. A., & Khatun, M. M. (2018). Isolation and identification of bacteria from fresh guava (Psidium guajava) sold at local markets in Mymensingh and their antibiogram profile. *Veterinary World*, *11*(8), 1145 – 1149

Satarug, S., Garrett, S.H., Sens, M.A. and Sens, D.A. (2010). Cadmium, environmental exposure, and health outcomes. Environment and Health Perspective. 118, 182-190.

Singh, A., Sharma, R., Agrawal, M., Marshal, F. (2010). Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. *Food and Chemical Toxicolology*. 48(2):611–19.

Sobukola, O. P., Adeniran, O. M., Odedairo, A. A., Kajihausa, O. E. (2010). Heavy-metal levels of some fruits and leafy vegetables from selected markets in Lagos, Nigeria. *African Journal of Food Science*. 4(2): 389 – 393.

Sue, R., Demand, R. D. (2015). Six primary nutrients provided by vegetables. Demend Media. RSS Feed. Healthy eating. Retrieved from sfgat.com. Retrieved on 13th August, 2015.

Taghipour, H., Mosaferi, M. (2013). Heavy Metals in Vegetables collected from Production Sites. *Health Promotion Perspectives* 3(2) 185-193.

Wang, X., Ouyang, Y., Liu, J., Zhu, M., Zhao, G., Bao, W., Hu, F.B. (2014). Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. *BMJ* 349: 1 – 14

World Health Organization (2000). https://www.euro.who.int/__data/assets/pdf_file/0017/123074/AQG2ndEd_6_4Chromium.PDF Date accessed 1st July, 2020

Zwolak, A., Sarzyńska, M., Szpyrka, E., & Stawarczyk, K. (2019). Sources of soil pollution by heavy metals and their accumulation in vegetables: A review. *Water, Air, & Soil Pollution*, *230*(7), 164 -.173.