Microbial and Nutritional Evaluation of Fresh and Wastewater Cultivated Cabbage in Quetta, Pakistan

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Received:- 07 May 2021/ Revised:- 18 May 2021/ Accepted:- 23 May 2021/ Published: 31-05-2021

Abstract— *Using wastewater for agricultural irrigation presents potential risks to public health and the environment. The aim of this study was to compare the microbial burden and nutritional components of cabbage grown in fresh and wastewater in Quetta, Pakistan. Cabbage samples randomly collected from fields irrigated with fresh and wastewater sources were analysed for viable bacterial and fungal counts, pH and vitamin contents. There was a significant difference in viable bacterial (3.0×10^6, 1.9×10^7 CFU g^{-1}) and fungal (2.4×10^3, 1.0×10^5 CFU g^{-1}) counts for cabbage grown in fresh and wastewater, respectively. There were no significant differences in pH (7.88/7.86) and contents of the vitamins thiamin (0.31/0.30 mg/100 g), riboflavin (0.05/0.04 mg/100 g), niacin (0.56/.54 mg/100 g), pyridoxine (0.13/0.12 mg/100 g) and ascorbic acid (57.00/56.60 mg/100 g). Results demonstrated that cabbage grown in wastewater had a significantly higher microbial burden than cabbage grown in fresh water, although there were no significant differences in nutritional components of cabbage from the different water sources. These observations emphasise the potential dangers to public health in using wastewater for agricultural irrigation.*

Keywords— Agricultural irrigation, Bacterial count, Brassica, Public health, Vitamins, Water resources.

I. INTRODUCTION

Vegetables have high water content and contain valuable nutrients such as carbohydrates, proteins, vitamins and minerals. These vital components are not only necessary for maintenance and buildup of the body but also in maintaining the alkaline reserve of the body (Hanif et al., 2006) and in repairing tissues and preventing various ailments (Bakhsh and Hassan, 2005; Lyaka et al., 2014; Lewu and Kambizi, 2015). An important *Brassica* vegetable is cabbage (*Brassica oleracea*), which is an herbaceous flowering plant with leaves forming a compact head and abundantly available in Pakistan for both human and animal consumption. Cabbage is low in calories and a good source of fiber, vitamin A, vitamin C, vitamin B_9, calcium and iron (Hossain et al., 2016). *Brassica* vegetables contain active ingredients such as 3,3-diindolylmethane, indole, isothiocyanates and dithiolthiones, which are reported to act as effective modulators to the inherent immune response, and effective anti-bacterial, anti-viral and anti-cancer agents (Zhang et al., 2014).

Consumption of green leafy vegetables has increased in recent years because of their nutritional significance and valuable health effects (Vandekinderen et al., 2008). Cabbage in Pakistan is mostly eaten raw as salad or added to fast foods such as burgers. It is also used as a cooked vegetable. Discarded cabbage as a vegetable by-product can be considered a potential non-conventional feed resource for ruminants. Cabbage may harbor a wide range of microbial contaminants, which undermine their nutritional and health benefits and may result in outbreaks of human infections from ingestion of fresh or minimally treated vegetables (Beuchat, 2002). Use of industrial and domestic effluents to irrigate agricultural land is a usual practice for wastewater disposal in Pakistan (Murtaza et al., 2010; Naz et al., 2016). Recurrent droughts, low rainfall and poorly performing irrigation structure increase water shortage and result in a widespread use of wastewater (Lone et al., 2000). Due to the presence of phosphorous and nitrogen, wastewater is a good source of plant nutrients, however (Nauman and Khan, 2010).

The current study was planned to compare influences of irrigation using fresh water (tube well) and municipal wastewater on the microbial loads and nutritional quality of cabbage.

II. MATERIALS AND METHODS

2.1 Sample collection

Cabbage samples (early cabbage, n=30, each ~1 kg) were randomly collected on three separate days (3 x 10 cabbages) from fields irrigated with fresh (tube-well) and wastewater sources, located near Smungli Road and Sabzal Road, Quetta. The samples were collected in pre-sterilized polyethylene plastic bags and immediately transferred to the Toxicology Laboratory of the Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB) for preparation and analysis of their viable bacterial and fungal counts, pH and vitamin contents.

2.2 Sample preparation and drying

On the same day as collection, cabbage samples were washed with filter-sterilised water (0.2 μM) and then cut into 10 mm thick transverse slices using a vegetable slicer. Subsequently, they were dried under shade conditions with a maximum day temperature of approximately 37 °C and a minimum night temperature of approximately 20 °C. The cabbage samples were weighed at various intervals over the entire drying period until their weights became constant. The dried cabbage slices were milled using a laboratory pestle and mortar and the powders were stored in pre-sterilized polyethylene bags at room temperature.

2.3 Sample analysis

Viable bacterial and fungal counts were determined following methods previously adopted by Abdullahi and Abdulkareem (2010) and Magnoli et al. (2002), respectively. To estimate the pH, juice from 10 g of each cabbage was blended using a National 4100 electric juicer and the pH was measured using a Jenway 3510 pH meter. The vitamin contents including vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_3 (niacin), vitamin B_6 (pyridoxine) and vitamin C (ascorbic acid) were determined by Syknm reversed phase (RP) isocratic HPLC equipped with an S1122 solvent delivery system and an S3210 UV-visible detector adopting the methodology of Sami et al. (2014). The solvents and water used for analysis were HPLC-grade and all of the vitamin standards were of chromatography grade.

2.4 Determination of vitamin contents

Cabbage powder (2 g) was placed in 0.1 N H_2SO_4 solution (made up to 25 mL) and incubated at 121 °C for 30 min. The contents were then cooled and the pH was adjusted to 4.5 by addition of sodium acetate (2.5 M). Samples were treated with the enzyme Takadiastase (50 mg) and then held overnight at 35 °C. The mixture was filtered using Whatman No. 4 filter paper, and then the filtrate was diluted with water (50 mL) and filtered using a 0.45 μm syringe. A 20 μL sample of the filtrate was injected into the HPLC instrument and quantification of vitamin B content was accomplished by comparing with vitamin B standards. Standard stock solutions of riboflavin, thiamine, pyridoxine and niacin were similarly prepared. Chromatographic separation was achieved using a mobile phase containing 33:67 $CH_3OH:H_3PO_4$ (0.023M, pH = 3.54) with a flow rate of 0.5 mL/min. The absorbance at 270 nm was recorded at room temperature.

2.5 Vitamin C analysis

Cabbage powder (10 g in triplicate) was blended and mixed with an extracting solution containing metaphosphoric acid (0.3 M) and acetic acid (1.4 M). The mixture was placed in a conical flask and agitated at 10,000 rpm for 15 min. The mixture was then filtered through a Whatman No. 4 filter paper. The vitamin C standard was prepared by dissolving vitamin C (100 mg) in extracting solution at a concentration of 0.1 mg/mL. Chromatographic separation was achieved on a RP-HPLC column through isocratic delivery of a mobile phase containing 50:50 CH_3CO_2K (0.1 M, pH = 4.9):CH_3CN with a flow rate of 1 mL/min. The absorbance at 270 nm was recorded at room temperature.

2.6 Data analysis

The data was statistically analysed with SPSS 16 for Windows using the independent sample student's t-test.

III. RESULTS

The results of the VBC and VFC tests of the cabbage samples are given in Table 1. The VBC values had ranges of 2.8×10^6 to 3.2×10^6 and 1.7×10^7 to 2.2×10^7 CFU g^{-1} (mean 3.0×10^6 and 1.9×10^7 CFU g^{-1}) for fresh and wastewater, respectively. The VFC values had ranges of 1.8×10^3 to 3.0×10^3 and 8.0×10^4 to 1.2×10^5 CFU g^{-1} (mean 2.4×10^3 and 1.0×10^5 CFU g^{-1}) for fresh and wastewater, respectively. There were significant ($P<0.05$) differences in the values of VBCs and VFCs in both fresh and wastewater.

The pH values of cabbage samples (Table 1) had ranges of 7.86 to 7.90 and 7.85 to 7.89 (mean 7.88 ± 0.02 and 7.86 ± 0.02) for fresh and wastewater, respectively. There was no significant ($P>0.05$) difference in pH between fresh and wastewater resources respectively. The measured pH values were close to those of Anon (1962).

TABLE 1
BACTERIAL, FUNGAL AND PH ANALYSIS OF CABBAGE SAMPLES

Parameter	Source of irrigation			
	Fresh water		Wastewater	
	Range	Mean ± SD	Range	Mean ± SD
VBC	2.8×10^6-3.2×10^6	$3.0 \times 10^6 \pm 1.6 \times 10^{5b}$	1.7×10^7–2.2×10^7	$1.9 \times 10^7 \pm 1.9 \times 10^{6a}$
VFC	1.8×10^3-3.0×10^3	$2.4 \times 10^3 \pm 492^b$	8.0×10^4 – 1.2×10^5	$1.0 \times 10^5 \pm 1.6 \times 10^{4a}$
pH	7.86-7.90	7.88 ± 0.02^a	7.85 - 7.89	7.86 ± 0.02^a

Mean values with the same letter in a row were not significantly (P>0.05) different. VBC = viable bacterial count, VFC = viable fungal count.

The contents of water-soluble vitamins (thiamine, riboflavin, niacin, ascorbic acid) are given in Table 2. Cabbage cultivated using fresh and wastewater irrigation contains 0.31/0.29 mg/100 g of thiamine, 0.05/0.04 mg/100 g of riboflavin, 0.56/0.54 mg/100 g of niacin, 0.13/0.12 mg/100 g of pyridoxine and 57.00/56.60 mg/100 g of ascorbic acid, respectively with no significant difference ($P>0.05$) for the two water sources.

TABLE 2
VITAMIN CONTENTS IN CABBAGE SAMPLES

Vitamin	Source of irrigation			
	Fresh water		Wastewater	
	Range	Mean ± SD	Range	Mean ± SD
B_1	0.27-0.36	0.31 ± 0.04^a	0.26-0.35	0.29 ± 0.04^a
B_2	0.03-0.06	0.05 ± 0.01^a	0.03-0.05	0.04 ± 0.01^a
B_3	0.44-0.69	0.56 ± 0.1^a	0.41-0.67	0.54 ± 0.1^a
B_6	0.12-0.15	0.13 ± 0.01^a	0.11-0.14	0.12 ± 0.01^a
C	55.00-59.00	57.00 ± 1.58^a	53.00-58.00	56.60 ± 2.07^a

Mean values with the same letter in a row were not significantly different (P>0.05). B_1 = thiamine, B_2 = riboflavin, B_3 = niacin, B_6 = pyridoxine.

IV. DISCUSSION

Cabbage irrigated with wastewater showed significantly higher microbial (viable bacterial and fungal counts) contamination. The findings regarding VBC are in agreement with Pesewu et al. (2014), Nwankwo et al. (2015), Dada and Olusola-Makinde (2015) and Hasibur et al. (2016), and the findings regarding VFC are in line with the results of Oluwafemi et al. (2013), Nwankwo et al. (2015) and Dada and Olusola-Makinde (2015). The presence of a higher microbial population in wastewater compared with fresh water is a natural phenomenon. The dairy and home sewage water, that is an integral part of wastewater in Quetta, is not only contaminated with microorganisms but also contains organic matter that serves as good culture media for microorganisms, especially bacteria and fungi (Alemayehu et al., 2015).

The current study revealed presence of appreciable quantities of vitamin C in the cabbage samples. Vitamin C helps in the formation of protein, collagen, bone, teeth, gums, cartilage, blood vessels, skin and scar tissue (Agea et al., 2014), facilitates the absorption of iron and calcium from the gastrointestinal tract, is involved in metabolism of fats and amino, increases resistance to infection and contributes to brain functioning (Schectman et al., 1991). The results were in line with the observations of Hanif et al. (2006), Mariga et al. (2012), Roe et al. (2013), Agea et al. (2014) and Ogbede et al. (2015). The recommended daily allowance (RDA) of vitamin C for a healthy adult is 65 to 90 mg, which could be easily attained by the use of cabbage as part of a low calorie diet (Agea et al., 2014).

V. CONCLUSION

The results from this study in Quetta, Pakistan demonstrated that cabbage grown in wastewater has a significantly higher microbial burden than cabbage grown in fresh water, although there were no significant differences in nutritional components of cabbage from the different water sources. These observations emphasise the potential dangers to public health in using wastewater for agricultural irrigation. Hence there is a need for regulatory authorities to ensure that wastewater is used for irrigation only after treatment. Further microbiological standards should be established and followed by agriculturalists and sellers for the handling and dispersal of such vegetables. End users are advised to thoroughly rinse such vegetables with clean water before use.

ACKNOWLEDGEMENTS

This work was funded by the University of Balochistan.

REFERENCES

[1] Abdullahi, I., & Abdulkareem, S. (2010). Bacteriological quality of some ready to eat vegetables as retailed and consumed in Sabon-Gari, Zaria, Nigeria. *Bayero Journal of Pure and Applied Sciences*, *3*(1), 173–175.

[2] Agea, J. G., Kimondo, J. M., Woiso, D. A., Okia, C. A., Obaa, B. B., Isubikalu, P., Okullo, J. B. L., Obua, J., & Teklehaimanot, Z. (2014). Proximate composition, vitamin C and beta-carotene contents of fifteen selected leafy wild and semi-wild food plants (WSWFPs) from Bunyoro-Kitara Kingdom, Uganda. *Journal of Natural Product and Plant Resources*, *4*(3), 1–12.

[3] Alemayehu, S., Dejene, T., Kiros, S., Mouz, M., & Equer, G. (2015). Microbial and parasitological quality of pre-harvest vegetables irrigated with urban wastewater and water from microdams in mekelle and southern zones of Tigray, region, Ethiopia. *Applied Journal of Hygiene*, *4*(2), 18–24.

[4] Anon. (1962). pH values of food products. *Food Engineering*, *34*(3), 98–99.

[5] Bakhsh, K., & Hassan, S. (2005). Use of sewage water for radish cultivation: A case study of Punjab, Pakistan. *Journal of Agriculture and Social Sciences*, *1*(4), 322–326.

[6] Beuchat, L. R. (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes and Infection*, *4*(4), 413–423.

[7] Dada, E. O., & Olusola-Makinde, O. (2015). Microbial and parasitic contamination on vegetables collected from retailers in main market, Akure, Nigeria. *American Journal of Microbiological Research*, *3*(3), 112–117.

[8] Hanif, R., Iqbal, Z., Iqbal, M., Hanif, S., & Rasheed, M. (2006). Use of vegetables as nutritional food, role in human health. *Journal of Agricultural and Biological Science*, *1*(1), 18–22.

[9] Hasibur, R., Farahnaaz, F., Kamal, K. D., & Rashed, N. (2016). Demonstration of the source of microbial contamination of freshly cultivated cabbage, cauliflower, potato and squash collected from rural farms of Bangladesh. *International Food Research Journal*, *23*(3), 1289–1295.

[10] Hossain, M. E., Ahmed, M. I., Sultana, S. A., & Karim, M. H. (2016). Kitchen waste-a promising feed resource for livestock. *Online Journal of Animal and Feed Research*, *6*(2), 30–37.

[11] Joshi, V. K., & Sharma, S. (2009). Lactic acid fermentation of radish for shelf stability and pickling. *Natural Product Radiance*, *8*(1), 19–24.

[12] Lewu, M. N., & Kambizi, L. (2015). Nutritional assessment of selected leafy vegetables. *International conference on biotechnology and food technology Harare*, Zimbabwe.

[13] Lone, M. I., Aslam, R., & Khan, K. S. (2000). Water quality and soil contamination in some industrial areas of Pakistan. *Pakistan Journal of Soil Science*, *18*, 1–6.

[14] Lyaka, Y. A., Idris, S., Alawode, R. A., & Bagudo, B. U. (2014). Nutrient content of selected edible leafy vegetables. *American Journal of Applied Chemistry*, *2*(3), 42–45.

[15] Magnoli, C., Chiacchiera, S. M., Miazzo, R., Palacio, G., Angeletti, A., Hallak, C., & Dalcero, A. (2002). The mycoflora and toxicity of feedstuffs from a production plant in Cordoba, Argentina. *Mycotoxin Research*, *18*(1), 7–22.

[16] Mariga, I. K., Mativha, L., & Maposa, D. (2012). Nutritional assessment of a traditional local vegetable (*Brassica oleracea var. acephala*). *Journal of Medicinal Plant Research*, *6*(9), 784–789.

[17] Murtaza, G., Ghafoor, A., Qadir, M., Owens, G., Aziz, M. A., Zia, M. H., & Saifullah. (2010). Disposal and use of sewage on agricultural lands in Pakistan: a review. *Pedosphere*, *20*(1), 23–34.

[18] Nauman, M., & Khan, K. S. (2010). Heavy metals contamination of soils in response to wastewater irrigation in Rawalpindi region. *Pakistan Journal of Agricultural Science*, *47*(3), 215–224.

[19] Naz, S., Anjum, M. A., & Akhtar, S. (2016). Monitoring of growth, yield, biomass and heavy metals accumulation in spinach grown under different irrigation sources. *International Journal of Agricultural Biology*, 18(4): 689–697.

[20] Nwankwo, I. U., Eze, V. C., Onwuakor, C. & Friday, J. U. (2015). Evaluation of the degree of contamination of salad vegetables sold in Umuahia main market. *American Journal of Microbiological Research*, *3*(1), 41–44.

[21] Ogbede, S. C., Saidu, A. N., Kabiru, A., & Busari, M. B. (2015). Nutrient and anti-nutrient compositions of *Brassica Oleracae Var. Capitata L*. *IOSR Journal of Pharmacy*, *5*(3), 19–25.

[22] Oluwafemi, F., Akisanya, E., Odeniyi, K., Salami, W., & Sharomi, T. (2013). Microbiological quality of street-vended foods and ready-to-eat vegetables in some Nigeria Cities. *African Journal of Biomedical Research*, *16*(3), 163–166.

[23] Pesewu, G. A., Gyimah, K. I., Agyei, J. N., Adjei, D. N., Olu-Taiwo, M. A., Asmah, R. H., & Ayeh-Kumi, P. F. (2014). Bacteriological assessment of the quality of *Brassica oleracea var. Capitata* grown in the Accra Metropolis, Ghana. *African Journal of Biomedical Research*, *8*(22), 2223–2228.

[24] Roe, M., Church, S., Pinchen, H., & Finglas, P. (2013). Nutrient analysis of fruit and vegetables. *Institute of Food Research*, Norwich Research Park, Conley, Norwich, Independent Nutritionist, Surrey, UK.

[25] Sami, R., Li, Y., Qi, B., Wang, S., Zhang, Q., Han, F., Ma, Y., Jing, J., & Jiang, L. (2014). HPLC analysis of water-soluble vitamins (B2, B3, B6, B12, and C) and fat-soluble vitamins (E, K, D, A, and β-carotene) of Okra (*Abelmoschus esculentus*). *Journal of Chemistry*, 831357, 6 pages. DOI: 10.1155/2014/831357.

[26] Schectman, G., Byrd, J. C., & Hoffmann, R. (1991). Ascorbic acid requirements for smokers: analysis of a population survey. *The American Journal of Clinical Nutrition*, *53*(6), 1466–1470.

[27] Vandekinderen, I., Van Camp, J., Devlieghere, F., Veramme, K., Denon, Q., Ragaert, P., & De Meulenaer, B. (2008). Effect of decontamination agents on the microbial population, sensorial quality, and nutrient content of grated carrots (*Daucus carota L.*). *Journal of Agricultural and Food Chemistry*, *56*(14), 5723–5731.

[28] Youseif, S. H., El-Halwagi, A., Sayed, H. A., & El-Itriby, H. A. (2014). Chemical analyses, antibacterial activity and genetic diversity assessment of some Egyptian Citrus spp. Cultivars. *African Journal of Biotechnology*, *13*(26), 2626–2636.

[29] Zhang, W. W., Feng, Z., & Narod, S. A. (2014). Multiple therapeutic and preventive effects of 3, 3′-diindolylmethane on cancers including prostate cancer and high grade prostatic intraepithelial neoplasia. *Journal of Biomedical Research*, *28*(5), 339–348.