ABSTRACT

Objective: A large number of farmers are growing different vegetables and supplying them to the Solapur market. The majority of these farms are located on the banks of the ‘nullah’, whose water is used for irrigation. From, a farm field in Degaon, a village on the Solapur–Mangalvedha road and on the bank of ‘nullah’ two fruit vegetables viz., Ladies Finger (Bhendi) and Cluster Beans (Gawar) are evaluated in the study. The purpose of this study was to analyse the presence of selected toxic non-essential heavy metals i.e. Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb) and Mercury (Hg) in the two vegetables.

Methods: Atomic Absorption Spectrophotometer (AAS) was used for determining the concentration of heavy metals. Optimised wet acid digestion procedure was employed to solubilize the metals from the vegetable samples.

Results: The following concentrations (mg/kg) of the non-essential heavy metals were found in the edible parts of the Ladies Finger and Cluster Beans, respectively: Ladies Finger: As (16.07), Hg (16.77), Pb (1.148), Cr (17.14), Cd (0.704 mg/kg) and Cluster Beans: As (18.76), Hg (21.86), Pb (1.675), Cr (17.14), Cd (0.888 mg/kg). The study revealed the presence of all the chosen NEHMs in the vegetables. Cadmium levels in both Cluster Beans and Ladies Finger were below the maximum acceptable limit and hence fit for human consumption as per FSSAI standards As (1.1), Pb (1.0), Cd (1.5), Hg (1.0 ppm). Though unsafe as per WHO/FAO standards As (0.1), Pb (0.5), Cd (0.2), Cr (1.2), Hg (0.05 ppm).

Conclusion: The other three NEHMs levels were approximately 15 times more than the permitted levels and hence might pose danger on human consumption.

Keywords: AAS, Cluster Beans, Grey water, Ladies Finger, Non-essential heavy metals, WHO/FAO, FSSAI

INTRODUCTION

Grey water is ‘Wash water’ or ‘Stormwater’ or ‘Wastewater’ or “Sewage water”. It is all waste water apart from toilet wastes and food wastes. There are a significant difference between greywater and toilet waste water; it is also called as ‘Blackwater’ [1-3].

Role of vegetables on health

Vegetables are the fresh and edible portions of herbaceous plants. They are essential food and highly beneficial for the maintenance of health and prevention of diseases. They contain valuable food ingredients like high carbohydrate, vitamin, mineral, fibre, antioxidants, metabolites and act as buffering agents for acidic substances which can be successfully utilized to build up and repair the body [4, 5]. The recent Joint FAO/WHO expert consultation on diet, nutrition and the prevention of chronic diseases, recommended the intake of a minimum of 400 g of fruit and vegetables per day (excluding potatoes and other starchy tubers) for the prevention of chronic diseases such as heart diseases, cancer, diabetes [6].

Heavy metals in vegetables and their toxicity

The process of absorption of inorganic substances depends on the pH and type of soil. It has been observed that the conc. of minerals vary from place to place and from plant to plant and on the source of water also [8, 9]. Non-essential heavy metals Cr, Cd, Pb, As, and Hg can get accumulated in commonly used vegetables i.e. Ladies Finger and Cluster Beans through the soil [10, 11].

Table 1: Permissible limits of heavy metals in vegetables as per WHO/FAO, FSSAI [7]

| Heavy metal | WHO/FAO permissible values (mg/kg) | Food safety and standards authority of India (FSSAI) parts per million (ppm) |
|-------------|-----------------------------------|--------------------------------------------------------------------------|
| As          | 0.1                               | 1.1                                                                      |
| Pb          | 0.5                               | 1.0                                                                      |
| Cd          | 0.2                               | 1.5                                                                      |
| Cr          | 1.2                               | -                                                                        |
| Hg          | 0.05                              | 1.0                                                                      |
Table 2: Symptoms due to different heavy metals toxicity [12]

| Heavy metals | Toxicity                                                                 |
|--------------|--------------------------------------------------------------------------|
| Arsenic      | Acute poisoning, Cancer of lungs, GI damage—severe vomiting, diarrhea    |
| Mercury      | Malfunctioning of nerves, kidneys and muscles, Neuromuscular changes (weakness, muscle atrophy, twitching), Emotional changes (mood swings, irritability, nervousness, etc.) |
| Cadmium      | Nephrotoxicity, stomach irritation resulting in vomiting and diarrhea     |
| Chromium     | Damage to DNA and proteins                                               |
| Lead         | Loss of appetite, head-ache, hypertension, abdominal pain, renal dysfunction, fatigue, sleeplessness, arthritis, hallucinations, vertigo. |

Ladies finger (LF) (Okra) (Bhendi)

'SF' contains vitamins and minerals as chief nutrients. It also contains a large amount of fiber, and has a unique profile of proteins. Okra mucilage has medicinal applications when used as a plasma replacement or blood volume expander [13].

Cluster beans (CB) (Guar) (gawar)

Cluster beans lower LDL, or bad cholesterol, levels thereby improving your heart health and lowering the risk of a heart attack. The iron in these beans increases body's hemoglobin production and allows blood to carry more oxygen around the body. Cluster beans have Vitamins A, B and K, in addition to minerals like calcium, iron, folate and potassium [14].

MATERIALS AND METHODS

Materials collection and location

Ladies Finger and Cluster beans were collected from a farm near Degaon (Degaon-Kegaon road) village at around 10 kms near Solapur on Solapur–Mangalveda road. (17.6721086 °N, 75.8489531 °E) [15].

They were ground by an electric grinder to small particle size and directly stored in fresh plastic containers for further analysis.

Methods

Wet acid digestion method as described by AOAC in 1990 was used in the study: 1.0 gm each of dried sample was added to 10 ml of conc. HNO₃ in 50 ml beaker and placed on the electric hot plate for 1 hour to get semi dried sample. Again 10 ml of conc. HNO₃ and 4 ml of H₂O₂ were added and again kept on a hot plate and heated vigorously. The addition of HNO₃ and H₂O₂ were continued till a colourless solution obtained. And its volume reduced up to 2-3 ml. It was cooled and filtered with the help of what man filter paper. The filtrate was stored in 10 ml sample bottles or vials. It was diluted up to 25 ml by de-ionized water before taking to Atomic Absorption Spectrometer. Metal contents in the prepared samples were analyzed using Atomic Absorption Spectrophotometer (ICE 3000, Thermo Scientific, USA) following the conditions described in AOAC (1990). Selected metals included Cadmium (Cd), Chromium (Cr), Arsenic (As), Lead (Pb), and Mercury (Hg). Calibrated standards were prepared from the commercially available stock solution in the form of an aqueous solution (1000 ppm). Highly purified de-ionized water was used for the preparation of working standards. All the glass apparatus used throughout the process of analytical work were immersed in 8N HNO₃ overnight and washed with several changes of deionized water prior to use.

RESULTS

Table 3: Comparative conc. Vs absorbance data for Cr in the standard and CB samples

| Sample ID | Absorbance | RSD % | Conc. mg/ml or µg/ml |
|-----------|------------|-------|----------------------|
| Cr Blank  | 0.001      | 43.6  | 0.0000               |
| Cr Standard 1 | 0.070 | 2.2   | 10.0000              |
| Cr-1      | 0.005      | 7.4   | 0.5868               |
| Cr-2      | 0.004      | 1.9   | 0.4500               |
| Cr-3      | 0.004      | 16.4  | 0.4243               |
| Cr-4      | 0.006      | 6.9   | 0.6799               |
| Cr-5      | 0.005      | 3.0   | 0.5931               |
| Cr-6      | 0.005      | 7.7   | 0.5692               |

Fig. 2: Location of nullah (Degaon-Kegaon road)

Fig. 3: Atomic absorption spectrophotometer (ICE 3000, thermo scientific, USA) (Solapur University, Solapur)
### Table 4: Comparative conc. Vs absorbance data for Cr in the standard and LF samples

| Sample ID | Conc. of HM | Absorbance | Rsd % | Conc. mg/l or µg/ml |
|-----------|-------------|------------|-------|---------------------|
| Cr-1      | 0.006       | 8.2        |       | 0.6823              |
| Cr-2      | 0.006       | 5.1        |       | 0.7763              |
| Cr-3      | 0.007       | 7.7        |       | 0.8323              |
| Cr-4      | 0.004       | 9.8        |       | 0.5214              |
| Cr-5      | 0.003       | 14.5       |       | 0.3659              |
| Cr-6      | 0.003       | 13.9       |       | 0.2970              |

#### Permissible limits

| As per WHO/FAO (mg/kg) | As per FSSAI (mg/kg) | CB     | LF     |
|------------------------|----------------------|--------|--------|
| 1.2                    | -                    | 17.142 mg/kg | 17.142 mg/kg |

### Table 5: Comparative conc. Vs absorbance data for As in the standard and CB samples

| Sample ID | Absorbance | Rsd % | Conc. mg/l or µg/ml |
|-----------|------------|-------|---------------------|
| As Blank  | 0.001      | 27.8  | 0.0000              |
| As Standard 1 | 0.373  | 0.8   | 40.0000            |
| As-1      | 0.010      | 10.6  | 1.1986             |
| As-2      | 0.008      | 4.3   | 0.9201             |
| As-3      | 0.007      | 8.0   | 0.8892             |
| As-4      | 0.008      | 5.4   | 0.9811             |
| As-5      | 0.005      | 2.2   | 0.6647             |
| As-6      | 0.005      | 26.4  | 0.6254             |

#### Permissible limits

| As per WHO/FAO (mg/kg) | As per FSSAI (mg/kg) | CB     | LF     |
|------------------------|----------------------|--------|--------|
| 0.1                    | 1.1                  | 18.766 mg/kg | 16.07 mg/kg |

### Table 6: Comparative conc. Vs absorbance data for As in the standard and LF samples

| Sample ID | Absorbance | Rsd % | Conc. mg/l or µg/ml |
|-----------|------------|-------|---------------------|
| As-1      | 0.002      | 53.7  | 0.3381              |
| As-2      | 0.007      | 7.3   | 0.8572              |
| As-3      | 0.009      | 3.2   | 1.0312              |
| As-4      | 0.009      | 3.0   | 1.0270              |
| As-5      | 0.007      | 3.0   | 0.8239              |
| As-6      | 0.007      | 6.2   | 0.8793              |

#### Permissible limits

| As per WHO/FAO (mg/kg) | As per FSSAI (mg/kg) | CB     | LF     |
|------------------------|----------------------|--------|--------|
| 0.2                    | 1.5                  | 0.888 mg/kg | 0.704 mg/kg |

### Table 7: Comparative conc. Vs absorbance data for Cd in the standard and CB samples

| Sample ID | Absorbance | Rsd % | Conc. mg/l or µg/ml |
|-----------|------------|-------|---------------------|
| Cd Blank  | 0.005      | 27.6  | 0.0000              |
| Cd Standard 1 | 1.360  | 0.2   | 10.0000            |
| Cd-1      | 0.006      | 12.8  | 0.0050              |
| Cd-2      | 0.005      | 12.9  | 0.0024              |
| Cd-3      | 0.004      | 14.5  | 0.0048              |
| Cd-4      | 0.005      | 5.0   | 0.0034              |
| Cd-5      | 0.005      | 7.2   | 0.0015              |
| Cd-6      | 0.004      | 6.7   | 0.0088              |

#### Permissible limits

| As per WHO/FAO (mg/kg) | As per FSSAI (mg/kg) | CB     | LF     |
|------------------------|----------------------|--------|--------|
| 0.2                    | 1.5                  | 0.888 mg/kg | 0.704 mg/kg |

### Table 8: Comparative conc. Vs absorbance data for Cd in the standard and LF samples

| Sample ID | Absorbance | Rsd % | Conc. mg/l or µg/ml |
|-----------|------------|-------|---------------------|
| Cd-1      | 0.004      | 10.3  | 0.0066              |
| Cd-2      | 0.004      | 2.3   | 0.0062              |
| Cd-3      | 0.003      | 5.8   | 0.0147              |
| Cd-4      | 0.001      | 13.1  | 0.0276              |
| Cd-5      | 0.000      | 12.2  | 0.0403              |
| Cd-6      | 0.011      | >99   | 0.1155              |

#### Permissible limits

| As per WHO/FAO (mg/kg) | As per FSSAI (mg/kg) | CB     | LF     |
|------------------------|----------------------|--------|--------|
| 0.2                    | 1.5                  | 0.888 mg/kg | 0.704 mg/kg |
Table 9: Comparative conc. Vs absorbance data for Pb in the standard and CB samples

| Sample ID | Absorbance | Rsd % | Conc. mg/l or µg/ml |
|-----------|------------|-------|---------------------|
| Pb Blank  | 0.000      | >99   | 0.0000              |
| Pb Standard 1 | 0.544     | 0.3   | 10.0000             |
| Pb-1      | 0.003      | 5.9   | 0.0556              |
| Pb-2      | 0.007      | 2.3   | 0.1366              |
| Pb-3      | 0.003      | 8.8   | 0.0575              |
| Pb-4      | 0.005      | 2.0   | 0.1028              |
| Pb-5      | 0.003      | 7.1   | 0.0543              |
| Pb-6      | 0.001      | 21.9  | 0.0307              |

Table 10: Comparative conc. Vs absorbance data for Pb in the standard and LF samples

| Sample ID | Absorbance | Rsd % | Conc. mg/l or µg/ml |
|-----------|------------|-------|---------------------|
| Pb-1      | 0.001      | 5.6   | 0.0091              |
| Pb-2      | 0.003      | 9.3   | 0.0614              |
| Pb-3      | 0.003      | 13.2  | 0.0604              |
| Pb-4      | 0.004      | 3.2   | 0.0832              |
| Pb-5      | 0.002      | 13.6  | 0.0451              |
| Pb-6      | 0.002      | 5.0   | 0.0469              |

Table 11: Comparative conc. Vs absorbance data for Hg in the standard and CB samples

| Sample ID | Absorbance | Rsd % | Conc. mg/l or µg/ml |
|-----------|------------|-------|---------------------|
| Hg Blank  | 0.107      | >99   | 0.0000              |
| Hg Standard 1 | 0.678      | 0.5   | 300.0000            |
| Hg-1      | 0.006      | 5.8   | 2.6232              |
| Hg-2      | 0.002      | 2.6   | 0.9867              |
| Hg-3      | 0.000      | 53.8  | 0.2661              |
| Hg-4      | 0.000      | 36.6  | 0.3035              |
| Hg-5      | 0.001      | 1.1   | 0.2302              |
| Hg-6      | 0.008      | 6.1   | 51.9592             |

Table 12: Comparative conc. Vs absorbance data for Hg in the standard and LF samples

| Sample ID | Absorbance | Rsd % | Conc. mg/l or µg/ml |
|-----------|------------|-------|---------------------|
| Hg-1      | 0.005      | 2.7   | 53.4478             |
| Hg-2      | 0.003      | 1.8   | 54.0988             |
| Hg-3      | 0.003      | 7.0   | 54.0740             |
| Hg-4      | 0.004      | 6.3   | 54.0163             |
| Hg-5      | 0.003      | 8.1   | 54.4573             |
| Hg-6      | 0.002      | 8.9   | 54.6968             |

Table 13: Consolidated list of conc. of HMs in CB and LF

| Metals | Heavy metals conc. in CB (Gawar) (mg/kg) | Metals | Heavy metals conc. in LF (Bhendi) (mg/kg) |
|--------|-----------------------------------------|--------|-----------------------------------------|
| As     | 18.77                                   | As     | 16.07                                   |
| Cd     | 0.89                                    | Cd     | 0.70                                    |
| Cr     | 17.14                                   | Cr     | 17.14                                   |
| Pb     | 1.68                                    | Pb     | 1.15                                    |
| Hg     | 21.87                                   | Hg     | 16.78                                   |

DISCUSSION

The AOAC method (1990) of estimation of heavy metals in organic samples using AAS yielded the results as presented in the above fig. and tables. From the results, following information could be derived:

Heavy metals conc. in cluster beans

Of the five NEHMs evaluated in CB all the five metals could be detected in the samples chosen. However, of the five, three heavy metals viz., As, Cr, and Hg were seen in exceedingly high conc. of 18.77, 17.14 and 21.87 mg/kg conc. respectively. Though, Cd (0.89 mg/kg) and Pb (1.68 mg/kg) were in relatively low conc. these two also were above their permissible limits of 0.2 and 1.0 mg/kg of the sample.

Heavy metal conc. in ladies finger

Of the five NEHMs evaluated in LF all the five metals could be detected in the samples chosen. However, of the five, three heavy metals viz., As, Cr, and Hg were seen in exceedingly high conc. of 16.07, 17.14 and 16.78 mg/kg conc. respectively. Though Cd (0.70 mg/kg) and Pb (1.15 mg/kg) were in relatively low conc. these two also were above their permissible limits of 0.2 and 1.0 mg/kg of the sample.

Permissible limits

| Metals | Heavy metals conc. in CB (Gawar) (mg/kg) | Metals | Heavy metals conc. in LF (Bhendi) (mg/kg) |
|--------|-----------------------------------------|--------|-----------------------------------------|
| As     | 18.77                                   | As     | 16.07                                   |
| Cd     | 0.89                                    | Cd     | 0.70                                    |
| Cr     | 17.14                                   | Cr     | 17.14                                   |
| Pb     | 1.68                                    | Pb     | 1.15                                    |
| Hg     | 21.87                                   | Hg     | 16.78                                   |
mg/kg) were in relatively low conc., these two also were above their permissible limits of 0.2 and 1.0 mg/kg of the sample.

CONCLUSION
The results indicate that all the non-essential heavy metals are chosen in this study viz., As, Cd, Cr, Hg and Pb were found to exist in both the vegetable samples. The concentration of As, Hg and Cr were found in excessively high amounts whereas, Cd and Pb were just above the permitted limits. This may be pointing towards possible contamination of ‘nullah water’ with wastes from automotive garages which might lead to elevated lead and mercury levels. But, it is interesting to see that the lead and cadmium conc. in both the vegetables were low indicating either inability of the plant to accumulate these NEHMs or their absence in soil/water. It is also interesting to note that the absorption pattern of all the five metals was similar in both CB and LF. This means the existence of an unfavourable set of conditions for the cultivation of these two vegetable crops. Further, consumption of CB and LF might also potentially exhibit toxicity symptoms of As, Cr and Hg on short-term treatment and Cd and Pb toxicity on long-term treatment. It might also indicate that the conditions of cultivation i.e., soil nature, water source are both unsuitable for the cultivation of the two plants. So, it might be better to substitute these two plants from others which don’t accumulate the NEHMs under study. Alternatively, it would also be possible to follow remediation of soil to avoid the uptake of HM by either changing their oxidation status or by changing soil pH.

ACKNOWLEDGEMENT
I am indebted to the rich source, deep inspire and my esteemed guide Dr. Belvotagi Venkatrao Adavirao, Associate Professor, Department Of Quality Assurance, D. S. T. S. Manda’s College of Pharmacy, Solapur. His words of advice have been etched in my heart and I always endeavour to hold up his ideas. It’s my pleasure to express sincere thanks to Prin. R.Y. Patil sir, Principal, D. S. T. S. Manda’s College of Pharmacy, Solapur and Dr. Kalshetti sir, HOD, Department of Quality Assurance, D. S. T. S. Manda’s College Of Pharmacy, Solapur. I am immensely thankful to, Dr. A. A. Ghanwat sir (Co-ordinator, Instrumentation centre), Dr. M.A. Kulkarni sir (Instrument Expert), R.C. Pawar sir (Laboratory Assistant) Solapur University, Solapur for assisting in Atomic Absorption Spectrophotometric measurements.

CONFLICT OF INTERESTS
Declare none

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How to cite this article
Doke Suraj Yashwant, Chavure Pranav Mohan, Belvotagi Venkatrao Adavirao. Grey water farming of ladies finger and cluster beans-induces dietary toxicity? Int J Curr Pharm Res 2017;9(4):104-108.