Effect of Heavy Metal Contaminated Waste Water Irrigation on Enzymatic and Non Enzymatic Antioxidants in Some Selected Vegetables

Jagdev Singh and Dr Anand Mohan

jagdevsingh5591@gmail.com

Department of Biosciences and Bioengineering, Lovely Professional University, Phagwara

Abstract

The study was conducted to investigate the bioaccumulation of heavy metals (As, Cd, Cr and Pb) and their effect on the antioxidant level and activity in vegetables Raphanus sativus, Spinacea oleracea, Brassica oleracea and Lycopersicum esculentum irrigated with heavy metal loaded waste water of Buddha Nullah. The results of the study revealed bioaccumulation of heavy metals in vegetables and the level of non-enzymatic antioxidants, ascorbic acid and proline and enzymatic antioxidants, catalase and peroxidase was found to be much higher in waste water irrigated vegetables in comparison to the level of bioaccumulation of heavy metals as well as antioxidants in same vegetables irrigated with normal borewell water as control. This indicates that heavy metals induce oxidative stress (ROS) in the plants at cellular level and to nullify the toxic effect these heavy metals the cell mechanism increase the production and activity of both non-enzymatic and enzymatic antioxidants.

Key Words; Heavy metals, antioxidants, waste water, vegetables

Introduction

Industrial and sewage waste water is frequently used by farmers to irrigate vegetable crops in peri urban areas (Feign et al 1991; Singh et al 2004). Sewage and industrial waste water is rich in nutrients like nitrogen, phosphorous, potassium and an appreciable amount of essential micronutrients like copper, zinc, manganese and nickel which play an important role in growth and metabolism of plant at lower concentrations. Presence of non-essential heavy metals like...
lead (Pb), arsenic (As), cadmium (Cd) and chromium (Cr) etc makes the water toxic. Vegetables irrigated with such a toxic waste water uptake the heavy metals which replace the essential micronutrients in the cell and alter the metabolic activities of the plant. (Nan et al 2002; Mapanda et al 2005).

Bioaccumulation of non essential heavy metals interferes with metabolic and physiological activities of the plant and induces stress conditions in the plant system. Heavy metals alter the shape and permeability of cell membranes, damage cell organelles and alter the physiological functions of plants leading to growth retardation (Rattan et al 2003). Plant produces reactive oxygen species such as hydrogen peroxide (H₂O₂), superoxide radical (O²⁻) and hydroxyl radicals (OH⁻) to nullify the heavy metal stress (Verma and Dubey 2001). Secondary metabolites and enzymatic antioxidants help the plant to scavenge the free radicals hence their survival under heavy metal stress, so to assess the stress responses in plant system, measurement of enzymatic and non enzymatic antioxidants is an essential aspect of biochemical studies (Gratao et al 2005).

Vegetables are an important part of our daily diet because of richness in essential nutrients like proteins, carbohydrates, vitamins, micronutrients, minerals, and fiber (Ullah et al 2009). Due to their short life span, perishable nature and nearby markets, marginal farmers tend to grow vegetables in peri-urban areas and irrigate them with waste water because of its nutritional richness and free availability throughout the hydrological year. Continuous use of heavy metal loaded waste irrigation leads to bioaccumulation of these toxic heavy metals in these vegetables which enters into food chain and poses many health hazards to the consumers (Sharma et al 2007).

Ludhiana is one of the most important industrial cities and is commonly referred to as Manchester of India. Buddha Nullah is a riverine passing through the city which daily receives about 500 million liters of sewage and 60 million liters of heavy metal rich industrial effluents from industries like dyeing, electroplating, chemical, pharmaceuticals and steel etc. This study was conducted from May 2017 to August 2019 to quantify the bioaccumulation arsenic (As), chromium (Cr), cadmium (Cd) and lead (Pb) in vegetables and enzymatic and non-enzymatic antioxidant levels in four different type of vegetables *Raphanus sativus, Spinacea oleracea, Brassica olerace and Lycopersicum esculentum* irrigated with waste water of Buddha Nullah.
and to compare the results with the same parameters of same vegetables grown and irrigated with normal borewell water 4 km away from the Buddha Nullah.

**Materials and methods**

Ludhiana (30.900965° N; 75.857277°E) has humid, sub-tropical climate, and on average observes 899 mm of precipitation annually. The temperature varies from 10°C to 40°C. Experimental area was the fields along the banks of Budha Nullah where irrigation was done with the waste water and the control area 4 km away from Budha Nullah where the vegetables were irrigated with borewell water.

**Sampling details**

Water samples were collected for physiochemical analysis from the Buddha Nullah which carries sewage and industrial waste water from different types of industries like dyeing, electroplating, chemical etc in sterilized glass bottles. Similarly soil samples were taken from both the sites (waste water irrigared and control) at a depth of 10 cm. Vegetable samples in triplicate were randomly collected from both the sites, sealed in sterilized plastic bags and refrigerated for biochemical tests. The vegetable samples collected were:
Radish (Raphanus sativus) Spinach (Spinacea oleracea) Cabbage (Brassica oleracea) and Tomato (Lycopersicum esculentum)

Analytical methods

Heavy metals analysis

Analysis of physiochemical parameters of waste and borewell water as well as carried out by methods given by (CPCB, 2011). Analysis of physiochemical parameters of soil samples was done by the methods (IS 2720) Wet digestion method was used to quantify the heavy metals in water, soil and vegetable samples by atomic absorption spectrophotometer (Alan Walsh et al, 1950).

Antioxidant enzymes activity

0.5 g fresh leaves of radish (Raphanus sativus), spinach (Spinacea oleracea), cabbage (Brassica oleracea) and tomato (Lycopersicum esculentum) were ground in 8ml of 50mM phosphate buffer, centrifuged at 15,000 × g at 4°C for 20 minutes. Peroxidase and catalase activity was measured by the method given by Chance and Maehly (1995).

Secondary metabolites (Non enzymatic antioxidants)

Estimation of proline content was determined by method given by Bates et al (1977) and Ascorbic acid was determined using the method given by Ranganna (1986).

Statistical analysis

All the data obtained was statistically analyzed. XLSTAT version 2008.5.01 software was used for analysis of the data of all the parameters explored.

Results

Physiochemical analysis of waste water

Table no. 1 and 2 represent the results of physiochemical analysis of the waste water of Buddha Nullah and borewell water which was used for irrigation of the vegetables at contaminated and control sites. The results were compared with Indian standards (IS ;1986) as well as borewell
water samples which reveal that the level of BOD, COD, Nitrate, Nitrite, Sulphate, Phosphate and toxic and non-essential heavy metals As, Pb, Cd and Cr were significantly higher as compared to the permissible levels in the waste water of Buddha Nullah. The pH of waste water was 7.9, slightly alkaline but lies within the normal values of the Indian standards as well as borewell water samples. Electric conductivity was recorded (2902µmoh) which is six times greater than that of borewell water.

**Table no. 1 Physiochemical analysis of waste water, Budda Nullah Ludhiana, Punjab**

Physical characteristics: Blackish, Purid and foul smelling

| S. No | Parameter                                | Unit  | Results       | Test method                      |
|-------|------------------------------------------|-------|---------------|----------------------------------|
| 1     | Chemical Oxygen Demand                    | mg/l  | 360 ± 0.6     | CPCB 14A                         |
| 2     | Bio Chemical Oxygen Demand                | mg/l  | 118 ± 0.49    | 10A                              |
| 3     | Nitrate (as NO$_3$)                       | mg/l  | 14.6 ± 0.78   | 20A                              |
| 4     | Nitrite (as NO$_2$)                       | mg/l  | BDL           | 21A                              |
| 5     | Sulphate (as SO$_4$)                      | mg/l  | 680 ± 0.16    | 23A                              |
| 6     | Phosphate (as PO$_4$)                     | mg/l  | 7.3 ± 0.20    | 18A                              |
| 7     | Arsenic (as As)                           | mg/l  | 0.08 ± 0.04   | Atomic absorption spectrophotometer |
| 8     | Cadmium (as Cd)                           | mg/l  | 0.98 ± 0.5    | Atomic absorption spectrophotometer |
| 9     | Chromium (as Cr)                          | mg/l  | 3.02 ± 0.6    | Atomic absorption spectrophotometer |
| 10    | Lead (as Pb)                              | mg/l  | 0.3 ± 0.01    | Atomic absorption spectrophotometer |
| 11    | Conductivity                             | mhos/cm | 2902 ± 17 | CPCB 112A                         |
12 Carbonate ml eq/l Nil 9A
13 Bicarbonate ml eq/l 11.2 ± 02 9A
14 Chloride ml eq/l 18.6 ± 09 27A
15 Calcium Magnesium ml eq/l 8.6 ± 0.8 9A
16 Residual sodium carbonate ml eq/l 5.2 ± 02 24A
17 pH ml eq/l 7.9 ± 01 6A

BDL means below detection limits

1. Results High levels of Nitrate, Phosphate, Sulphate and Conductivity(ions
2. Presence of heavy metals above the standard detection limits
3. Not fit for agricultural irrigation

Table no. 2 Analysis of Physiochemical parameters of Borewell Water (Control Site)

Physical characteristics colorless and transparent

| S.No | Parameter                  | Units | Results   | Test method              |
|------|----------------------------|-------|-----------|--------------------------|
| 1    | Nitrate (as NO₃⁻)         | mg/l  | 0.09      | CPCB 20A                 |
| 2    | Nitrite (as NO₂⁻)         | mg/l  | 0.08      | 21A                      |
| 3    | Sulphate (as SO₄²⁻)       | mg/l  | 17.6      | 23A                      |
| 4    | Phosphate (as PO₄³⁻)      | mg/l  | 0.08      | 18A                      |
| 5    | Arsenic (As)              | mg/l  | BDL       | Atomic absorption        |
|      |                            |       |           | spectrophotometer         |
| 6    | Cadmium (Cd)              | mg/l  | BDL       | Atomic absorption        |
|      |                            |       |           | spectrophotometer         |
| 7    | Chromium (Cr)             | mg/l  | BDL       | Atomic absorption        |
|      |                            |       |           | spectrophotometer         |
| 8    | Lead (Pb)                 | mg/l  | BDL       | Atomic absorption        |
|      |                            |       |           | spectrophotometer         |
Detection limits for heavy metals: As = 0.01, Cd = 0.01, Cr = 0.02, Pb = 0.01
BDL means below detection limits

Table no. 3 and 4 represents the comparison of the physiochemical parameters of the soil irrigated by waste water to the soil irrigated by borewell water and it was concluded that there is not much difference in pH value of both the soil samples (7.4 for waste water irrigated and 7.2 for borewell irrigated soil). A significant difference in the heavy metal content of both the soil samples was recorded. Arsenic (As) was found to be 16 ± 0.4 mg/kg in waste water irrigated soils whereas its amount was much lower 3.2 mg/kg in borewell irrigated soil samples. Similarly both the soils significantly differ as far as the amount of chromium (Cr) is concerned. Chromium was found to 18.20 ± 0.6 mg/kg in waste water irrigated soils whereas 6.9 mg/kg in borewell irrigated soil samples. There was not much difference in concentration of cadmium (Cd) and lead (Pb) in both the soil samples. A significant difference was recorded in electric conductivity in soil samples, which was 1830 mhos/cm in contaminated soil as compared to 580 mhos/cm in the control site soil. The level of heavy metals and electric conductivity was found to be much higher than the standard permissible limits (WHO, 2007).

Table no. 3: Showing analysis of soil sample (Buddha Nullah site).

| S. no. | Parameter          | Unit      | Results          | Atomic absorption spectrum |
|-------|--------------------|-----------|------------------|-----------------------------|
| 9     | Carbonate          | millieq/l | Nil              | CPCB 9A                     |
| 10    | Bicarbonate        | millieq/l | 16.2 ± 0.01      | 9A                           |
| 11    | Chloride           | millieq/l | 1                | 27A                          |
| 12    | Calcium magnesium  | millieq/l | 14.2 ± 0.01      | 9A                           |
| 13    | Residual sodium    | millieq/l | 10.9 ± 0.6       | 24A                          |
| 14    | Carbonate          | mhos/cm   | 536 ± 0.16       | 12A                          |
| 15    | pH                 |           | 7.4 ± 0.4        | 6A                           |
### Table 4: Physiochemical Analysis of Soil Samples (Borewell Site)

| No. | Element          | Unit | Value   | Method            |
|-----|------------------|------|---------|-------------------|
| 1.  | Arsenic (As)     | mg/kg| 16 ± 0.4| Atomic absorption spectrum |
| 2.  | Cadmium (Cd)     | mg/kg| 5.8 ± 0.3| Atomic absorption spectrum |
| 3.  | Chromium (Cr)    | mg/kg| 18.20 ± 0.6| Atomic absorption spectrum |
| 4.  | Lead (Pb)        | mg/kg| 9.30 ± 0.4| Atomic absorption spectrum |
| 5.  | Zinc (Zn)        | mg/kg| 4.2 ± 0.06| IS 2720 |
| 6.  | Iron (Fe)        | mg/kg| 25.3 ± 0.6| IS 2720 |
| 7.  | Manganese (Mn)   | mg/kg| 8.2 ± 10 | IS 2720 |
| 8.  | Copper (Cu)      | mg/kg| 3.60 ± 0.8 | IS 2720 |
| 9.  | Conductivity (2:1 of slurry) | μmoh/cm | 1830 ± 12 | IS 2720 |
| 10. | pH               |      | 7.4 ± 0.08 | IS 2000 |

*Values with Mean SE of three replicates are significantly different (p< 0.05)*

*Electric conductive many folds above the permissible levels*
| S. no. | Parameter            | Unit     | Results     | Test method                      |
|--------|----------------------|----------|-------------|----------------------------------|
| 1.     | Arsenic (As)         | mg/kg    | 3.2 ± 0.8   | Atomic absorption spectrum       |
| 2.     | Cadmium (Cd)         | mg/kg    | 4.10 ± 0.12 | Atomic absorption spectrum       |
| 3.     | Chromium (Cr)        | mg/kg    | 6.90 ± 0.6  | Atomic absorption spectrum       |
| 4.     | Lead (Pb)            | mg/kg    | 6.2 ± 0.8   | Atomic absorption spectrum       |
| 5.     | Zinc (Zn)            | mg/kg    | 4.10 ± 0.04 | IS 2720                          |
| 6.     | Iron (Fe)            | mg/kg    | 16.10 ± 0.8 | IS 2720                          |
| 7.     | Manganese (Mn)       | mg/kg    | 4.8 ± 0.4   | IS 2720                          |
| 8.     | Copper (Cu)          | mg/kg    | 3.2 ± 0.8   | IS 2720                          |
| 9.     | Conductivity (2:1 of slurry) | µmoh/cm | 580 ± 0.04 | IS 2720                          |
| 10.    | pH                   |          | 7.2 ± 0.06  | IS 2000                          |

Values with Mean SE of three replicates are significantly different (p< 0.05)

**Bioaccumulation of heavy metals in vegetables**

**Table no 5** represents bioaccumulation of all the four heavy metals studied arsenic (As), cadmium (Cd), chromium (Cr) and lead (Pb) in the waste water and borewell irrigated vegetable samples. It was found that bioaccumulation of all the 4 heavy metals studied in all the 4 vegetables was much higher than the permissible levels (WHO/FAO, 2001) in vegetables samples from contaminated site as compared to the control site ones. Minimum amount of arsenic (As) was observed in *Brassica oleracea* (0.004 mg/kg). Cadmium (Cd) accumulation was recorded maximum in *Raphanus sativus* and *Lycopersicum esculentum* to be 11.6 mg/kg and 12 mg/kg respectively. Biaccumulation of chromium (Cr) was also found to be much higher in waste water irrigated vegetables and maximum amount was observed in root vegetable *Raphanus sativus* (29.6±0.06 mg/kg).
Tables No 5. : Effects of waste water & borewell water irrigation on heavy metal absorption of vegetables

| Name of the Vegetable | Arsenic (mg/kg) | Cadmium (mg/kg) | Chromium (mg/kg) | Lead (mg/kg) |
|-----------------------|----------------|----------------|-----------------|--------------|
| Radish (w/w)          | 0.008 ± 0.003  | 11.80 ± 0.6    | 29.6 ± 0.06     | 7.10 ± 0.4   |
| Radish (b/w)          | Nil            | 2.0 ± 0.002    | 12.6 ± 0.08     | 1.80 ± 0.02  |
| Spinach (w/w)         | 0.012 ± 0.002  | 6.80 ± 0.001   | 11.0 ± 0.4      | 1.6 ± 0.02   |
| Spinach (b/w)         | Nil            | 4 ± 0.2        | 10.60 ± 0.02    | 3 ± 0.001    |
| Cauliflower (w/w)     | 0.004 ± 0.004  | 11 ± 03        | 16 ± 04         | 3 ± 0.02     |
| Cauliflower (b/w)     | Nil            | 2 ± 0.02       | 7 ± 0.02        | 1.20 ± 0.002 |
| Tomato (w/w)          | 0.005 ± 0.001  | 12 ± 0.8       | 16 ± 0.07       | 3 ± 0.04     |
| Tomato (b/w)          | Nil            | 2 ± .02        | 6 ± 0.03        | 1 ± 0.01     |

Values with Mean ± SE of three replicates are significantly different (p< 0.05)

Table no. 6 : Showing permissible levels of heavy metals in vegetables (FAO/WHO,2001)

| Heavy Metals   | Vegetable (mg/kg) |
|----------------|-------------------|
| Arsenic (As)   | --                |
| Cadmium        | 0.10              |
| Chromium (Cr)  | 0.50              |
| Lead (Pb)      | 0.50              |

Table no. 7 summarizes the bioaccumulation of non-antioxidants (secondary metabolites) in both waste water irrigated and borewell irrigated vegetable samples. The results show a significant higher accumulation of both the secondary metabolites analyzed in vegetables samples of Budha Nullah site as compared to the vegetables samples collected from control site. Maximum amount of ascorbic acid was observed as 55±0.80 and 50±0.2 mg/100g in Raphanus sativus and Spinacea oleracea respectively. Proline content was found to be much higher in
Brassica oleracea (1.8 ± 0.004 mg/100 g) and minimum 0.8 ± 0.001 in Lycopersicum esculentum samples collected from waste water irrigated site

Table No.7: Amount of Ascorbic Acid and Proline in vegetables (g/100 gm fresh weight)

| Name of the Vegetable | Ascorbic Acid (mgVCE/100g) | Proline Content (mg/100g) |
|-----------------------|-----------------------------|---------------------------|
| Radish (b/w)          | 45 ± 0.12                   | 1.0 ± 0.002               |
| Radish (w/w)          | 55 ± 0.18                   | 1.5 ± 0.001               |
| Spinach (b/w)         | 42 ± 0.7                    | 1.4 ± 0.001               |
| Spinach (w/w)         | 50 ± 0.12                   | 1.1 ± 0.003               |
| Cauliflower (b/w)     | 30 ± 0.4                    | 1.1 ± 0.002               |
| Cauliflower (w/w)     | 38 ± 02                     | 1.8 ± 0.004               |
| Tomato (b/w)          | 35 ± 0.3                    | 0.8 ± 0.001               |
| Tomato (w/w)          | 44 ± 0.8                    | 1.4 ± 0.003               |

w/w : Waste Water irrigated b/w : Borewell Water irrigated

Values with Mean ± SE of three replicates are significantly different (p< 0.05)

Activity of antioxidant enzymes

The activity of both the antioxidant enzymes, catalase (Graph no 1) and peroxidase (Graph no 2), was found to be significantly higher in all the vegetable samples irrigated with waste water as compared to the borewell water. Maximum activity of catalase 1.45µ/mg protein was recorded in leafy vegetable Spinacea oleracea. Peroxidase activity was also found to be much higher in Spinacea oleracea as compared to other vegetables (1.65µ/mg protein).
Graph no. 1 showing effect of waste water and borewell water on activity of catalase

Graph no. 2 showing effect of waste water and borewell water on activity of peroxidase
Discussion

Continuous and long term waste water irrigation contributes significantly to the bioaccumulation of heavy metals in soil and vegetables as compared to normal borewell water (Mapanda et al 2005; Sharma et al 2007) which poses serious health concerns. The heavy metal accumulation in leafy vegetables has been reported to be manifold high as accumulated by other type of vegetables (Sinha et al 2006). These toxic heavy metals disturb the physiological and biochemical activities of plants leading to their stunted growth (Singh and Agrawal, 2007). The results of present study show a significant increase ascorbic acid amount in all the vegetables samples collected from contaminated site where the vegetables were irrigated with heavy metal loaded waste water. Ascorbic acid is known to be an active antioxidant which plays a protective role against metal stress (Guo et al 2005). Different vegetables produce different amount of ascorbic acid when exposed to the same stress conditions is related to the fact that depending upon various factors the bioaccumulation of various pollutants differ from species to species (Barman and Lal, 1994). Similarly waste water irrigated vegetables exhibit high amount of proline content as compared to control. High accumulation of proline has been reported in roots of *Brassica napus* when exposed to increased concentration of lead (Gohari et al 2012). A significant increase in activity of both the antioxidant enzymes catalase and peroxidase reveals that production of these enzymes might be playing an important role to scanvage the ROS produced in response to bioaccumulation of heavy metals in the cell. These enzymes are known to play a defensive role against oxidative stress (Radotic et al 2000). The increase in activity of antioxidant enzymes relates to the similar study conducted by Singh and Agarwal (2007) in which they reported a significant increase in the activity of peroxidase and catalase in *Beat vulgaris* irrigated with different concentrations of sewage waste water.

Conclusion

The outcome of present study revealed that the vegetables irrigated with waste water tend to bioaccumulate heavy metals much higher than the permissible levels and induces a kind of stress at cellular level. To cope up with this heavy metal induced stress, the production of various secondary metabolites and activity of antioxidant enzymes is increased by the plant. But the bioaccumulation of heavy metals varies from species to species hence the activity of antioxidant enzymes and amount of various secondary metabolites produced is related with the amount of
heavy metals absorbed by the plant. It was concluded that as compared to root and fruit vegetables, leafy vegetables biaccumulate significantly higher amounts of toxic heavy metals. So, keeping in mind the health issues of consumers, raising of leafy vegetables must be restricted in such heavy metal contaminated areas along the waste water channels and it should be mandatory that all the industries should abide the laws laid by pollution control board before throwing the pollutants rich effluents in such nullahs. Awareness of ill consequences of wastewater irrigation among farmers and consumers is need of the hour.

Acknowledgement

The authors are thankful to Lovely Professional University, for providing laboratory facilities

Conflict of Interest

No conflict of interest is reported

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