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Comparative Analysis of Heavy Metal Profile of *Brassica campestris* (L.) and *Raphanus sativus* (L.) Irrigated with Municipal Waste Water of Sargodha City

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Abstract

The recent study was carried out for the comparative analysis of heavy metals in *Brassica campestris* and *Raphanus sativus* irrigated with municipal waste water of Sargodha city. Field surveys were done for this experiment. Three experimental sites i.e Bhalwal road, Ajnala road and Faisalabad road were selected in Sargodha city for field surveys. Vegetables samples were collected from these sites which were grown in municipal waste water. These Samples were used for the analytical comparison of heavy metals i.e Copper, Chromium, Lead, Cadmium, Nickel, Zinc, Cobalt, Arsenic, Manganese, Iron, Magnesium and Molybdenum, accumulated in *Brassica campestris* and *Raphanus sativus* at three life stages.

Keywords: Comparative; Municipal; Chromium; Arsenic; Accumulated

Introduction

There is water shortage in the country and farmers use waste water to irrigate their vegetable fields. These irrigation practices give very good crop yields because waste water contains large amounts of organic material and some inorganic elements essential for plant growth. But it may also contain large amounts of non-essential heavy metals which can be transferred to animal and human beings through food chain [1].

Pakistan is an agrarian country of Southeast Asia. Increased urbanization and industrialization have resulted in discharge of effluents of toxic nature, polluting the water bodies and making them unfit for consumption in agriculture sector [2].

Heavy metals are generally present in agricultural soils at low levels but due to their cumulative behavior and toxicity they have potential hazardous effects on plants and human health [3].The main problem is increasing amount of heavy metals in our environment by industrialization use of fertilizers in agricultural and domestic activities, this leads harmful effects on the human beings and ecosystem [4].

Farmers use wastewater for irrigation with an objective of being a rich source of nutrient and economically sufficient, as it saves a lot of fertilizer costs [5] particularly in agricultural lands located near cities or in the vicinity of an industrial area [1].

Continuous irrigation with wastewater could lead to the accumulation of heavy metals in soils beyond crop tolerance levels [6]. The uptake of metals from the soil depends on different factors such as their soluble content in it, soil pH, plant growth stages types of species, fertilizers and soil [7]. Consequently, the usage of wastewater for irrigation ends to soil contamination and heavy metals accumulation both in soil and crops [8,9].

Uptake of trace metals from soil differs from plant to plant and from site to site. Thus, there is need of careful studies in order to fully analyze and understand the long term environmental impacts of the use of sewage water for crop irrigation [10]. The impact of anthropogenic activities on urban and suburban soil has also increased. The original structure and properties of soil have been deeply modified, and new soils with particular characteristics, have been created [11].

Waste water has deleterious effects on soil and it cannot be properly used for agricultural practices due to salinity and sodicity problems which impose harmful effects on seedlings of plants [12]. Most of the leafy vegetables which were grown in contaminated soil accumulate higher amount of heavy metals in their leaves [13].

Plants grown in contaminated soils or irrigated with municipal wastewater when consumed by people can result in health problems like diarrhea, mental retardation, liver and kidney damage [14]. The practice of using industrial and sewage wastewater has become common to irrigate fields in countries which are developing [15-18].

The widespread heavy metal contamination has raised public and scientific interest hence special attention is given to them throughout the world due to their effects even at very low concentrations [19]. Industrial and domestic liquid wastes are frequently channeled either into the same sewerage system (if a sewerage system exists) or into the same open drains. The number and types of livestock the household owns will influence the type of wastewater-related activities in which they engage [20].

Dietary intake of heavy metals through contaminated vegetables may cause various diseases. Heavy metal concentrations such as Cd, Cu, Zn and Pb in surface soils have been a focus of investigation over the past decade [21].

The aim of this research work is to investigate the heavy metal profile of *Brassica campestris* and *Raphanus sativus* irrigated with municipal wastewater of Sargodha city.

Materials and Methods

Collection of samples

The vegetables (*Brassica campestris* and *Raphanus sativus*) samples were randomly collected from the fields irrigated with municipal wastewater of Sargodha city at three growth stages (seedling, vegetative and maturity stages). Leafy vegetables were preferred for sampling because previous researches indicate that they accumulate heavy metals at a greater capacity than other vegetables [13].

Study area

Three different sites were selected to determine the sewage water effects on vegetables

i) Bhalwal road, ii) Ajnala road, iii) Faisalabad road

Washing of samples

The collected vegetable samples were washed with distilled water to remove dust particles. The samples were then cut to separate the roots/stems and leaves. After washing the fresh weight of samples were taken and then places in an oven at 100°C for one week to dry samples. When samples dried these were ground into fine powder, stored and used for acidic digestion.

Preparation of samples/Wet digestion

Samples (0.2 g) of leaves of each vegetable were weighed on electric balance and treated with 10 ml of concentrated HNO_3. A sample was prepared applying 10 ml of HNO_3 into empty digestion flask. The flasks were kept for a night at room temperature.

After that the samples were digested against 2 ml H_2O_2 on Hot plate. H_2O_2 was further added to the sample (2 ml of each was added occasionally) and digestion continued until a colorless solution was obtained.

After cooling, the solution was filtered with Whatman No. 42 filter paper and it was then transferred quantitatively to a 50 ml volumetric flask by adding distilled water up to 50 ml volume. Soil digestion was also carried out in the same way as above. Water was filtered and then subjected to analysis.

Preparation of standards and analysis of samples

Working standard solutions of Lead (Pb), Copper (Cu), Chromium (Cr), Zinc (Zn), Cadmium (Cd), Iron(Fe), Arsenic (As), Cobalt (Co), Aluminum (Al), Mercury (Hg),Manganese (Mn), Nickel (Ni), Magnesium (Mg) and Molybdenum (Mo) were prepared from the stock standard solutions containing 1000 ppm of element and measurement of elements were done on atomic absorption spectrophotometer.

The calibration curves were prepared for each element individually applying linear correlation by least square method. A blank reading was also taken and necessary correction was made during the calculation of concentration of various elements.

Statistical analysis

Three samples of leaves of each vegetable were analyzed individually. Data were reported as significant and non-significant. One way SPSS analysis of variance (ANOVA) was used to determine significant difference between groups (Tables 1 and 2).

Parameters	Seedling stage	Vegetative stage	Maturity stage
Mn	0.000***	0.000***	0.000***
Fe	0.000***	0.000***	0.000***
Cd	0.000***	0.000***	0.001**
Co	0.003**	0.003**	0.000***
Zn	0.001***	0.004***	0.006***
Ni	0.001**	0.003**	0.003**
Mg	0.003**	0.000***	0.004**
Cu	0.000***	0.004**	0.008**
Cr	0.001**	0.000***	0.006**
Pb	0.000***	0.002**	0.002**
As	0.000***	0.000***	0.000***
Hg	0.000***	0.000***	0.000***
Mo	0.001**	0.000***	0.000***
Al	0.000***	0.000***	0.001**

Table 1: Heavy metal profile of *Brassica campestris* at three growth stages. The table shows p-values, results are significant at ($p<0.05$-$P<0.001$), where *=0.05, **=0.01 and ***=0.001.

Parameters	Seedling stage	Vegetative stage	Maturity stage
Mn	0.000***	0.000***	0.000***
Fe	0.000***	0.000***	0.000***
Cd	0.000***	0.002***	0.004***
Co	0.000***	0.000***	0.000***
Zn	0.002***	0.003***	0.004***
Ni	0.000***	0.001**	0.002**
Mg	0.000***	0.000***	0.000***
Cu	0.123	0.000***	0.000***
Cr	0.000***	0.000***	0.000***
Pb	0.000***	0.000***	0.000***
As	0.000***	0.000***	0.000***
Hg	0.000***	0.000***	0.000***
Mo	0.123	0.001**	0.000***
Al	0.000***	0.000***	0.000***

Table 2: Heavy metal profile of *Raphanus sativus* at three growth stages. The table shows p-values, results are significant at ($p<0.05$-$P<0.001$), where *=0.05, **=0.01 and ***=0.001

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Results and Discussion

The surveyed data showed variation in heavy metals concentrations of different water sources, soil and vegetable samples. All the sewage water samples by which vegetables were irrigated showed the safe limits of heavy metals accumulation. The field experimental data showed that due to sewage application, Zn content was much higher in leaves of *Brassica campestris* ranging from 0.001 to 0.006 $mg.Kg^{-1}$. Cadmium accumulation in the vegetables irrigated with sewage water was also much higher ranging from 0.001 to 0.004 $mg.Kg^{-1}$. Nickel also showed the similar trend for its accumulation in the vegetables ranging from 0.001 to 0.002 $mg.Kg^{-1}$.

Rests of the elements are not toxic to human unless they are present in high concentrations. The present study provides baseline data on trace metal concentrations of Lead (Pb), Copper (Cu), Chromium (Cr), Zinc (Zn), Cadmium (Cd), Iron (Fe), Arsenic (As), Cobalt (Co), Aluminum (Al), Mercury (Hg),Manganese (Mn), Nickel (Ni), Magnesium (Mg), and Molybdenum (Mo), in *Brassica campestris* and *Raphanus sativus*, their proximate analysis and nutritional composition (Figure 1).

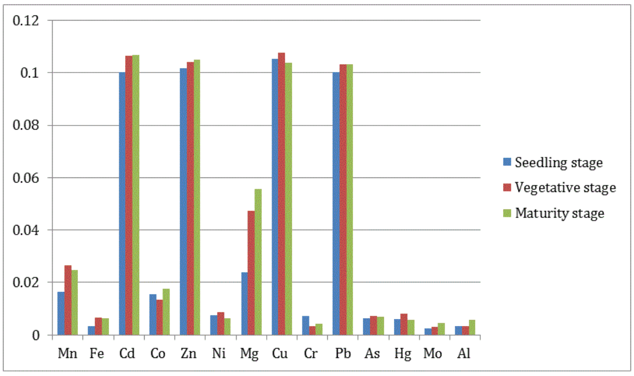

Figure 1: Concentration of heavy metals in the leaves of *Brassica campestris* at three different growth stages ($mg.kg^{-1}$).

Manganese (Mn) Concentration was maximum at vegetative stage and minimum concentration was examined at initial stage of *Brassica campestris*. Iron (Fe) concentration in *Brassica campestris* was minimum in seedling stage but nearly equal at vegetative and maturity stage. Cadmium (Cd) concentration in *Brassica campestris* was maximum at maturity stage but minimum at seedling stage. Cobalt (Co) concentration in *Brassica campestris* leaves was maximum at maturity stage and minimum at vegetative stage. Zinc (Zn) concentration in *Brassica campestris* leaves was in order as maturity>vegetative>seedling. Nickel (Ni) concentration in *Brassica campestris* was maximum at vegetative stage but minimum at maturity stage. Magnesium (Mg) concentration in *Brassica campestris* leaves was in order as maturity>vegetative>seedling. Copper (Cu) concentration in *Brassica campestris* was maximum at vegetative stage but minimum at final stage. Chromium (Cr) concentration in *Brassica campestris* leaves was maximum at seedling stage but minimum at vegetative stage. Lead (Pb) concentration in *Brassica campestris* leaves were minimum at seedling stage and almost equal at vegetative and maturity stage. Arsenic (As) concentration in *Brassica campestris* leaves was maximum at vegetative stage and minimum at seedling stage.

Mercury (Hg) concentration in *Brassica campestris* was maximum at vegetative stage but minimum at maturity stage. Molybdenum (Mo) concentration in *Brassica campestris* leaves was in order as maturity>vegetative>seedling. Aluminium (Al) Concentration was maximum at maturity stage but nearly equal at seedling and vegetative stage.

Mostly higher concentration was examined at maturity stage which may be due to Bioaccumulation or Biomagnification. These results are in accordance with results of [22]. Some of these metals are even not essential for plant growth and after accumulating in the soil could be transferred to food chain (Figure 2).

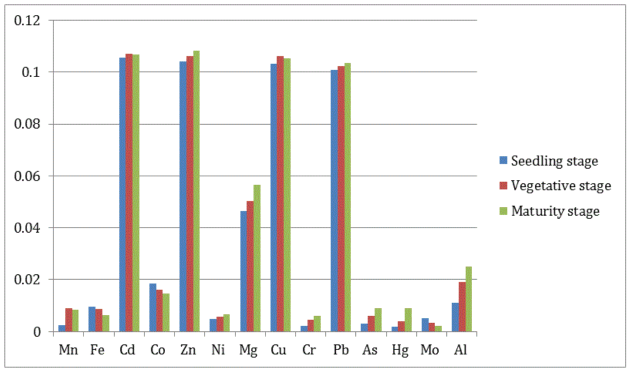

Figure 2: Concentration of Heavy metals in Root at 3 different growth stages of *Raphanus sativus* ($mg.kg^{-1}$).

Concentration of Mn was maximum at vegetative stage and minimum concentration was examined at initial stage. Fe concentration in *Raphanus sativus* was maximum at seedling stage but minimum at final stage. Cd concentration in *Raphanus sativus* was maximum at maturity stage but minimum at seedling stage. Co concentration in *Raphanus sativus* leaves was maximum at seedling stage and minimum at final stage. Zn concentration in *Raphanus sativus* leaves was in order as final>middle>initial. Ni concentration in *Raphanus sativus* was maximum at maturity stage but minimum at seedling stage and intermediate at middle stage. Mg concentration in *Raphanus sativus* leaves was in order as middle>final>initial. Cu concentration in *Raphanus sativus* was maximum at vegetative stage but minimum at initial stage. Cr concentration in *Raphanus sativus* leaves was minimum at initial stage but maximum at maturity stage. Pb concentration in *Raphanus sativus* leaves was in order as maturity>vegetative>seedling. As concentration in *Raphanus sativus* leaves was in order as maturity>vegetative>seedling. Hg concentration in *Raphanus sativus* was maximum at maturity stage but minimum at seedling stage. Mo concentration in *Raphanus sativus* leaves was in order as seedling> vegetative>maturity. Al Concentration was maximum at maturity stage and minimum concentration was examined at seedling stage.

Concentrations of metals vary greatly at three different stages of studied vegetables. Same results were reported by Y. Latif [23]. Soils irrigated with wastewater had higher concentrations of metals as compared to canal or underground water/other sources. However, the concentration of metals varied with soil texture, depth and concentration of metals in polluted water [23].

Conclusion

The survey data revealed variation in heavy metals concentrations of different water sources. All water samples in sewage water irrigated vegetables were within the safe limits. As comparison the leaves of *Brassica campestris* accumulated higher concentration of heavy metals at three different growth stages while the roots of *Raphanus sativus* have least significant lower concentration of heavy metals. The field experimental data showed that due to sewage application, Zn content was much higher in leaves of *Brassica campestris*. Cadmium accumulation in the vegetables irrigated with sewage water was also much higher. Like all the heavy metals, Nickel also showed the similar trend for its accumulation in the vegetables.

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