METAL ENRICHMENT IN WHEAT IRRIGATED WITH INDUSTRIAL EFFLUENT, CANAL AND TUBEWELL WATER OF GUJRANWALA, PAKISTAN

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A thesis submitted to Bahria University, Islamabad in partial fulfillment for the degree of M.S in Environmental Policy and Management

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ABSTRACT

The current study was carried out to investigate the heavy metal content of Pb, Cr, Hg, As and Cd in wheat samples irrigated with industrial effluent, tubewell and canal water of Gujranwala, Pakistan and associated human health risk. From different agricultural fields of Gujranwala, a total 45 samples of wheat were randomly collected. Out of those 45 samples, 8 samples were collected from sites irrigated with industrial effluent, 16 samples collected were irrigated with canal water and 21 samples collected that were irrigated with tubewell water. According to the results obtained from current study, concentration of heavy metals in wheat samples were estimated in order to calculate non-carcinogenic (HQ) and carcinogenic risks (CR) of the heavy metals for adults and children following the risk assessment modals proposed by the US-EPA. High concentration of heavy metals i.e. Pb, Cr, Cd and Hg were found in wheat samples irrigated with industrial effluent than canal and tubewell water whereas, Arsenic shows high concentration in samples of wheat that were irrigated with tubewell water than industrial effluent and canal water. The translocation factor in wheat irrigated with industrial effluent, canal, and tubewell showed general trend as Pb > Hg >Cd > Cr >As. DIE values calculated for all studied metals revealed that Pb, Cr and Cd have high DIE in wheat consumed irrigated with industrial effluent in adults whereas in children, Pb show highest DIE value. HQ estimated for non-carcinogenic risk was found to be higher for Hg and Cd as compared to Pb, As and Cr. Whereas, the carcinogenic risk for adults and children in wheat are with safe limit (1E-06 to 1E-04) of US-EPA (2011). However, if heavy metals contaminated food is continuously consumed may result into potential buildup of harmful and various disorders in humans. Therefore, long-term monitoring and more studies are obligatory to keep check and balance for the safety of humans under such conditions.

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Approval for Examination

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Contents

CHAPTER 1

INTRODUCTION

In the 20th century, due to anthropogenic activities level of pollution has increased through discharge of large amount of wastewater accompanied with toxic heavy metals (Khan et al., 2013). Most serious environmental concerns in current times is the heavy metal accumulation because they cause toxicity in crops as well as potentially harmful for animals and humans. These metals are major environmental pollutants resulting in cytotoxic, mutagenic and carcinogenic effects in humans and animals (More et al., 2003; Al-Othman et al., 2011).

A well-known source of irrigation water is surface water of streams and rivers in Pakistan which is either diverted or pumped into nearby agricultural fields (Qadir et al., 2008). Among other possible sources of such contamination are excessive wastewater irrigation practices (Singh et al., 2010). The industrial, domestic and urban wastewaters discharge into the surface water without prior treatment is prominent. Moreover, various other wastewater sources including oil and ghee industries, sports industries, tanneries and cutlery industries wastes, while electroplating, coloring, metal surface treatment, fabric printing, batteries and paints manufactories, surgical instruments and garages wastes are also found to be the major contributors. Same wastewater bodies are used for the purpose of irrigation (Khan et al., 2013).

Over a century, wastewater that is used for urban areas agriculture has become a general trend mostly in water scanty areas. As a result, heavy metals started to accumulate in plants and soils (Ugulu et al., 2012). Due to presence of organic matter and nutrients deposition in wastewater, is considered as a rich for irrigation (Ping et al., 2011). Food crops are adversely affected because of continuous practice of wastewater irrigation (Hua et al., 2013). The most of the irresistible metals combined in the organic environment in various ways through the food chain. Thus, plants grown in a polluted environment can accumulate trace elements at high concentrations and long term exposure to these metals via food consumption can cause lethal impacts on human (e.g., heart, kidney, brain, and bone diseases) (Alloway et al., 1990; Jarup, 2003; Hussain et al., 2014). A wide range of toxic chemicals, especially heavy metals (HMs) are found in food crops (Dorne and Fink Gremmels, 2013; Khan et al., 2013 a, b). That is why 30% of human cancers are caused due to high level of contaminated food (Mansour et al, 2009).

In many countries, major source of food are the cereals which includes; Wheat (Triticum sp.), corn (Zea mays), barley (Hordeum vulgare), oat (Avena sativa), rice (Oryza sativa) and rye (Secale cereale), making the human diet nutritious. But among these cereals most widely spread and consumed is wheat (Araujo et al., 2007). As a result, highly contaminated wheat can create potential health risk due to its high consumption (Doe et al., 2013).

1.1 Essential and Non Essential Heavy Metals

Heavy metals are of two types: one is micronutrients including iron, zinc, copper, molybdenum and manganese and are essential for human body as well as plant growth (Penney et al., 2004). Other type is macronutrients including chromium, nickel and lead are nonessential for both plants and animals and if present in high concentration in both plants and animals can cause severe problems (Mapanda et al., 2007). Non-essential heavy metals are found in traces, but during irrigation when industrial effluents and municipal waste water is used is taken up by crops and make it part of the organic phenomenon (Vaca-Paulin et al., 2006; Yusufa et al., 2003). The presence of those non-essential heavy metals in soil is usually considered as a possible hazard and need monitoring soil additionally as crop grown on soil (Nafees and Amin, 2014).

1.2 Sources of Heavy Metals

Sources of heavy metals in the natural environment are of two types. One is the mobilization of HM below natural prerequisites (intrinsic factor), such as geogenic factors, weathering reactions, natural biological activity, geochemical reactions, and volcanic emissions. Other sources of heavy metals are anthropogenic sources (extrinsic factor), such as mining activities, fossil fuels combustion, high pesticides use in agriculture, and additives in cattle feed (Sun et al., 2010). If high concentrations of HMs are brought about with the aid of anthropogenic activities, it is crucial to take movements to improve the supervision of contamination sources (Wang et al., 2010).

There are additionally many factors which make a contribute to heavy metal contamination such as contaminated irrigation water, fertilizers and pesticides applications, emissions of various waste materials from industries, lack of good transportation facilities, harvesting procedure and storage (Afzal et al., 2012). Heavy metals pose a threat to the environment due to their toxicity (Sardans et al., 2011). The accumulation of heavy metals in soil adversely affects its physicochemical properties leading to infertility and low yield of crops (Khan et al., 2009). Exposures to higher levels of HMs were resulted from the industrial waste and emission such as cement, brick kiln, electroplating, Fe and steel, ghee mills and tanneries. Therefore, results were further evaluated for the potential non-carcinogenic and carcinogenic risks (Lu et al., 2014, Chen et al., 2015).

1.3 Wheat: A Vital Source of Food

Agricultural commodities such as fruits, vegetables, and cereals (wheat) are significant component of our daily diet and act as a buffering agent during digestion process of food in human body. Wheat is an important food-crop cultivated worldwide with production of about 725 million tons in 2015 and is a staple food in many countries. The average per-capita consumption of wheat in developed and developing countries were 95 and 61 kg, respectively, with worldwide average of 67 kg in 2015 (FAO, 2016). In Pakistan, per-capita wheat utilization is about 124 kg year−1, as 72% daily body caloric requirements are accomplished with the intake of wheat products, much higher than the average per-capita consumption of other countries (OECD/FAO, 2015). Like other plants, when grown in contaminated soils, wheat has natural potential to absorb non-essential elements along with essential ones and ultimately transfer them to food web (Peralta-Videa et al., 2009).

Wheat is one of the main crops and an integral constituent of the national diet. It plays a vital role in human growth by providing carbohydrates, proteins and certain inorganic micronutrients (Anita et al., 2010). If accumulation of metals in grain is under the permissible limits then it is safe to consume wheat in daily food (Das, 1990; Khan et al., 2008). However, when accumulations exceed the permissible limit, it exerts toxic effects and may produce a variety of diseases in human (Melamed et al., 2003; Al-Othman et al., 2012b).

Wheat (*Triticum aestivum L.*) is the most vital crop worldwide, followed by coarse grains and rice. In Pakistan, wheat is a staple grown across some 8,069,000 ha. According to the Pakistan Agricultural Research Council (PARC), per capita wheat consumption of the country is 120 kg per year – among the highest in the world (PARC, 1989). Due to more consumption of highly contaminated wheat can create potential health risk (Doe et al., 2013).

High concentrations of heavy metals like Zn, Pb, Cd, Ni, and Cr usually found in top soil that is irrigated wastewater (Mishra et al., 2009).

1.4 Toxic Heavy Metals and Their Health Impacts

Heavy metals viz., Cadmium (Cd), Lead (Pb), Chromium (Cr), Nickel (Ni), Arsenic (As), and Mercury (Hg) are bioaccumulative and persistent in nature (Muhammad et al., 2011). These metals may produce some adverse impacts via the food chain on long-term exposure in humans (e.g., heart, kidney, brain, and bone diseases). Furthermore, some heavy metals such as Cd and Pb may cause mutagenesis, teratogenesis, and carcinogenesis (Jarup 2003; Hussain et al., 2014). Elevated blood pressure and renal infection, improper hemoglobin synthesis, and damage to the reproductive system are caused by lead accumulation in humans. Manganese may cause Parkinson's disease as result of iron oxide deposition (Harmanescu et al., 2011). Zinc and Cu can cause toxicity in humans and animals if concentrations of these metals exceed above recommended levels in food (Kabata-Pendias and Mukherjee, 2007). Arsenic exposure causes a markedly elevated risk for developing a number of cancers, most notably skin cancer and cancers of the liver, lung, bladder, and possibly the kidney and colon (Morales et al., 2000).

1.5 Irrigation System in Pakistan

In Pakistan about two thirds of the population depending on the agriculture sector for their livelihood. Regional soil fertility and quality directly leads to an increase in agricultural production. But for this reason, agricultural soils of Pakistan are facing an ever-increasing pressure not only to ensure sustainable food supplies to rapidly expanding population that is over 160 million now but also to support the livelihood of two thirds of the population (Ali et al. 2014). In Pakistan, agricultural fields are mostly irrigated with different sources of water including industrial effluent without bothering about their quality and quantity. Even though industrial effluent is a good alternative of fresh water and its application has many advantages in agriculture but it is a potential source of heavy metal contamination. This results in elevated concentration of metals and other pollutants in surface horizons (Ghafoor et al. 1995; Hussain et al. 2006).

Various researchers have documented higher metal loads in the agricultural soils in response to wastewater irrigation practices. Mushtaq et al. (2010) investigated heavy metal contents in the Rawalpindi soils irrigated with municipal and industrial wastewaters. They reported total metal levels of Cd, Ni, and Fe which exceeded threshold concentrations in investigated arid soils. Agricultural soils are subjected to elevated levels of heavy metals in the urban and peri urban areas of Sialkot, Gujranwala, Lahore, Kasur, Peshawar, Karachi due to complex anthropogenic activities (Midrar-ul-haq et al., 2003; Tariq et al., 2005; Tariq et al., 2006; Malik et al., 2010; Mushtaq et al., 2010; Ali et al., 2015b).

1.6 Problem Statement

In Pakistan, most of the population depends upon agriculture to meet food needs and mostly agriculture fields are irrigated with one of these methods such as canals, tubewells and industrial wastewater. But due to scarcity of water level in Pakistan, usually farmers irrigate their fields with industrial wastewater which consist of huge amount of heavy metals that is discharged by the industries. Main reason of increased level of non-essential heavy metals in Pakistan is that the municipal waste water and industrial effluent are drained directly into rivers, streams and irrigation canals without preceding treatment which leads to accumulation in the river, canal bed or reach to agriculture field in Pakistan (Nafees and Amin, 2014).

The study was carried out in the Gujranwala district, Pakistan, which has large number of industries including tanneries, cutlery, pharmaceutical, electroplating, surgical instrument, ceramics, sanitary, utensils, steel, metal tools, and textiles. These industries discharge large concentration of wastewater and solid waste into the environment. Fertilizers and pesticides are also utilized on the agricultural lands to enhance crops' productivity. Moreover, wastewater irrigation is a common practice in urban and peri-urban areas. Due to lack of monitoring, rules and regulations, and policies have further intensified the problem of food contamination (Khan et al., 2015).

1.7 Significance of the Study

Heavy metals pollution impose risks causes various environmental issues like poor harvests, decreased soil fertility, microbial activity and leads to cause major toxic effects on human health (Durkan et al., 2011). The primary pathway of human exposure to heavy metals is the consumption of crops, vegetable and fruit irrigated with contaminated water and grown on contaminated soil. After having entered the soil, heavy metals tend to accumulate in animal tissues via consumption of contaminated vegetables. Therefore, it is important to define the content of heavy metal accumulation in plants like fruits, crops and vegetables, and related researches have gained increasing attention (Ugulu et al., 2016).

In the current study, wheat samples were collected from the different locations of Gujranwala district, Pakistan in order to analyse heavy metal content in theses samples and then compared with the FAO/WHO standards. The main purpose of this study was to monitor whether wheat crops irrigated with wastewater, canal and tubewell water is suitable for consumption or not if heavy metals content in those samples are above permissible limits and also to evaluate the health risk associated with consumption of wheat.

1.8 Aim and Objectives of the Study

"The main aim of the study is to assess the concentration of toxic heavy metals viz cadmium (Cd), chromium (Cr), nickel (Ni), arsenic (As), mercury (Hg) and lead (Pb) in wheat crops (*Triticum aestivum*) irrigated with three different sources of water i.e wastewater, canal and tube well in Gujranwala district, Pakistan and compare the results with the standards and estimate the human health risk".

The specific objectives of the study are:

1. To evaluate the concentration of toxic heavy metals such as cadmium (Cd), chromium (Cr), nickel (Ni), arsenic (As), mercury (Hg) and lead (Pb) in wheat crops (grains and straws) irrigated with industrial wastewater, canal and tube well in different areas of Gujranwala district, Pakistan.
2. To assess the human health risk associated with consumption of contaminated wheat crops in order to better understand how metals are transported and accumulate through the food chain.

Chapter 2

METHODOLGY

This section outlines all the methods, equipment and procedures that were used to carry out research project on the assessment of heavy metals in wheat crops and its risk on human health in Gujranwala district, Pakistan. The following heavy metals Pb, Cr, Cd, Hg, and As were considered to be analysed in wheat samples.

2.1 Study Area

Gujranwala is the 6th largest city (World Gazetteer, Retrieved 22 August 2012) of Pakistan. It has a population of about 2,661,360 as of 24 June 2011. Gujranwala is located at the latitude of 32.16° north, and longitude of 74.18° east and is 226 metres (744 ft) above sea level. Extensive road and rail links help the city to flourish in agriculture and manufacturing markets. It is in between Lahore, Gujrat and Wazirabad. It is situated on GT road so an easily connection to Islamabad, Lahore and Peshawar. Gujranwala is the fastest growing cities of Pakistan. Gujranwala exports variety of products which includes sugarcane, world's finest quality of rice melons and grains. This city contributes to 9% in national production and 8% in revenue generation (Kanwal, 2015).

The district consists of four tehsils: Gujranwala, Kamoke, Nowshera Virkan and Wazirabad. District is situated on the Grand Trunk Road (GT Road), built in 16th Century by the Emperor Sher Shah Suri (FAO.org). Gujranwala city is connected with Lahore and Peshawar via rail and GT Road. Gujranwala city is very important from the point of view of industrial activities, and is designated as 5th largest city of the Punjab and 7th amongst the big populous cities of Pakistan. Gujranwala contribute for 9 percent in national production and 8 percent in the revenue. The study area hosts several commercial and industrial centers for the manufacturing of ceramics, metal tools, leather, utensils, fans, textiles, pipe fittings, agricultural tools etc (Dawn, 2006).

The soil of Gujranwala is alluvial and fertile supporting a good amount of agriculture. The main crops grown in the district are rice, wheat and sugarcane. The agriculture fields are irrigated by perennial and nonperennial canals supplemented by tub wells. Rice and Wheat are major food crops while Rice dominates with anearnings of about Rs.15 million as foreign exchange. Other than these crops, Mash, Jawar, Masoor, Bajra, Moong, Gram, Maize, Tobacco, Oil Seed such as Rape/Mustard and sunflower are also grown in minor quantities in the district. Main fruits grown in the district are Guavas and Citrus. Besides these two fruits, some other fruits in minor quantities such as Peaches, Jaman and Banana are also grown. The major vegetables grown in the district are Potatoes, Cauliflower, Turnip, Onion, Peas, Carrot, Tomato, Okra and Garlic. Besides, some vegetables in minor quantities such as Bitter Gourd and Chillies are also grown in the district.

Gujranwala is located at the gps coordinates of (31◦ 32’ –32◦ 33’ latitudes N and 73◦ 11’ –74◦ 28’ longitudes E) has a population of 3 million. This city hosts a large number of industries: tanneries, ceramics, cutlery, pharmaceutical, electroplating, surgical instrument, utensils, steel, metal tools, sanitary and textiles. Huge volume of wastewater and solid waste is released by these industries into the environment. In urban and peri-urban areas, wastewater irrigation is a common practice. But due to lack of monitoring, rules and regulations, and policies have further aggravated the problem of food contamination (Khan et al., 2014). Following is the map showing areas where wheat samples were collected from peri urban areas of Gujranwala, Pakistan in fig 2.1.

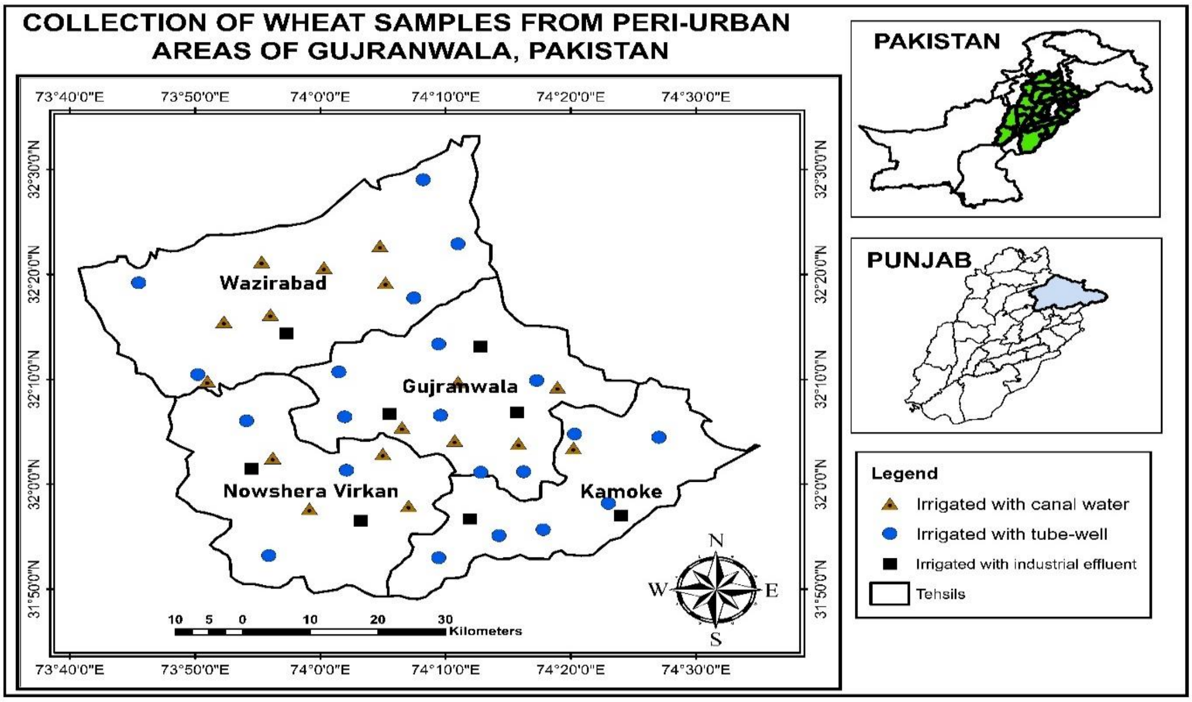

Figure.2.1 Maps Showing Study Area where Wheat Samples were Collected in Peri Urban Areas of Gujranwala.

2.2 Sample Collection

To study the heavy metals contents in wheat and associated health risks, wheat plant samples were randomly collected from farmer fields of Gujranwala irrigated with three different sources of water: industrial effluent, tubewell, and canal water. Total 45 samples of wheat were randomly collected from different agricultural fields in Gujranwala. Out of those 45 samples, 8 samples were collected from sites irrigated with industrial effluent, 16 samples collected were irrigated with canal water and 21 samples collected that were irrigated with tubewell water. All samples were collected and saved in airtight polythene bags and brought to the soil chemistry lab of Land Resource Research Institute (LRRI) located at National Agriculture Research Centre (NARC), Islamabad to determine heavy metal concentrations in order to compare them with WHO/FAO standards and to assess the human health risk.

2.3 Sample Preparation

Firstly, all the samples collected were rinsed with tap water in order to remove any adhered dust and soil particles, thereafter, washed with the deionized distilled water (DDW) (Khan et al., 2014). After washing, samples were subjected to oven drying at 70-80˚C for 24 hours (Khan et al., 2013). Then the straws and grains were manually separated from the dried samples of wheat. After separation, 90 samples of wheat (45 straw and 45 grain samples) were grounded to fine powder using an electric grinding machine and then samples were sieved with a sieve (2 mm) and again stored in plastic bags at room temperature before analysis.

Acid wash was prepared in tub by mixing 15000 ml of water and 75 ml of sulphuric acid (H_2SO_4) in order to thoroughly rinsed glassware viz., digestion flasks, volumetric flasks, and plasticware viz., funnels and storage bottles used for digestion and making volume. It was necessary to wash every glassware before digestion to remove any source of contamination and error in results. After dipping glassware in acid wash for 24 hours, they were then washed with tap water followed by washing with de-ionized distilled water. All glassware were then oven dried at 80˚C and storage bottles were dried at 25-30˚C till fully dried. Proper numbering and identities were given to all samples such as samples taken from first location of waste water was written as1S and 1G whereas replicates were also made and they were written as 1S(R) and 1G (R). Here, S stands for wheat's straw, G stands for wheat's grain and R stands for replicate of actual sample. Samples from second location were written as 2S,2S(R), 2G and 2G(R) and all samples were numbered following this sequence.

2.4 Heavy Metals Analysis of Wheat

2.4.1 Wet Acid Digestion of Wheat Samples

Wet acid digestion was carried out afterwards for wheat samples in order to prepare them for heavy metal analysis. For selected heavy metals in wheat samples, 1 g of over dried, grounded wheat samples were digested with 15ml mixture of nitric acid (HNO_3), sulphuric acid (H_2SO_4) and perchloric acid ($HCLO_4$) in 5:1:1 v/v. Samples were heated upto 100˚C on hot plate until the solution gets transparent. Digested samples were then cooled and filtered using funnel and Whatman filter paper No. 42. Then, volume was raised up to the 50 ml mark with distilled water, these extracts were stored in plastic bottles at 40˚C and labelled accordingly to be used for further analysis of total concentration of heavy metals i.e. Pb, Cr, Cd, Hg and As. Blank solution (without addition of food crop sample) was also prepared as per above mentioned digestion method. (USEPA Method: 3005A).

2.4.2 Determination of Heavy Metals (Pb, Cr, Cd, As and Hg)

For quality data assurance, each sample was analyzed in replicates (90 samples with 90 replicates). Total concentration of Pb, Cd and Cr were analyzed by using Graphite Furnance (Perkin elemer AAnalyst 700). The standards solutions were prepared from stock metals standards in order to calibrate the instrument and to check its performance standards were run on instrument for each metal. The standards of lead (Pb), chromium (Cr) and cadmium (Cd) were prepared using $C_1V_1= C_2V_2$ formula, in which C_1 is the known concentration of available solution, V_1 is the volume to be found, C_2 is the concentration of new solution to be made and V_2 is the volume of new solution to be made. Standard stock solutions (1000 mgL^{-1}) of Pb, Cd and Cr metals were used to prepare sub stock and working solution of 5,10,20,40,80 and 100 ugL^{-1} using distilled water (DDW) and working solutions were used to calibrate the curve. In order to determine Pb, Cr and Cd, blank solution and each sample was then loaded onto machine plate and it picks blank solution first and then each sample one by one and analyse metal content in it. Results were monitored on screen attached with furnace. After getting results, all the readings were first subtracted by the reading of blank solution and then multiplied by dilution factor which was 50ml and then results were divided by 1000 to convert them into mgL^{-1} from ugL^{-1}.

Mercury (Hg) and arsenic (As) were determined using Mercury/Hydride System (MHS) (Perkin elemer AAnalyst 700). The Mercury/Hydride System is used for the analysis of high-sensitive elements like mercury (Hg) and hydride-forming elements, such as As, Se, Sb, Te, Bi and Sn. It is one of the convenient technique because it has less operating costs and minimum capital investment and offers the best possible detection limits, down to the nanogram (ng) range. The technique has been proven in tens of thousands of laboratories worldwide as it is simple to use, easy-to-handle, and highly consistent technique (www.perkinelmer.com).

Standard solutions (1000 mgL^{-1}) of Hg and As were used to make sub stock and working solution of 20,40,60,80 and 100 ugL^{-1} using hydrochloric acid (HCL). Before sample analysis, 10 ml of HCL was run through machine and then working standards in order to calibrate the curve. Samples and blank were prepared by pouring 9 ml of HCL and 1ml of sample into tubes and 1 drop of potassium permanganate ($KMNO_4$) was added to each sample. Then prepared samples were run through machine after sample blank to analyse Hg and As metal content and results were monitored on screen attached with machine. Tubes were washed with HCL after discarding analysed sample to avoid contamination. Final results were then calculated by subtracting blank's reading from evaluated readings and multiplied them with dilution factor of 50 and then 10. After that, all the readings were converted to ppm from ppb by dividing them with 1000.

2.4.3 Chemical Preparation for MHS

Chemicals were also prepared for the analysis of Hg and As including; Hydrochloric acid (HCL), Sodium hydoxide (NaOH) and Sodium Borohydride ($NaBH_4$).

Hydrochloric Acid (HCL) Preparation

1.5 % of hydrochloric acid (0.15M) was prepared in 5 litres of a beaker, first pour 2 litres of distilled water in it and then measure 75 ml of hydrochloric acid in a measuring cylinder and pour it into beaker filled with distilled water and raised its volume upto the mark of 5 litres. hydrochloric acid was used as a stabilizer for washing tubes and making standards and samples.

Sodium Hyroxide (NaOH) Preparation

1% of sodium hyroxide (0.25M) was prepared by measuring 10 g of sodium hyroxide using scale. Then, add some distilled water in 1000ml volumetric flask and add measured NaOH in flask and mix it thoroughly until sodium hyroxide dissolves in it. After mixing, raised its volume upto 1000 ml. Now, prepared sodium hyroxide was used for making sodium borohydride.

Sodium Borohydride ($NaBH_4$) Preparation

Sodium borohydride solution was prepared for MHS in solution of 300 ml of NaOH solution which was prepared earlier. 9 g of $NaBH_4$ was weighed on a scale and 100 ml of NaOH was measured using measuring cylinder and poured into glass beaker. Then, 9 g of $NaBH_4$ was also added to glass beaker and now remaining 200 ml of NaOH was poured into beaker to make a solution of $NaBH_4$. The solution was then filtered through nitro cellulose filter using vacuum generator because solution was not 100% pure. Then, filtered solution was poured into $NaBH_4$ bottle and attached it to the MHS.

Before sample analysis, 10 ml of HCL was run through machine in order to stabilize it and then standards of 20 ppb, 40 ppb, 60 ppb and 80 ppb were run on machine by pouring 5 ml of HCL and 5ml of standard into tubes. Samples and blank were prepared by pouring 9 ml of HCL and 1ml of sample into tubes and 1 drop of potassium permanganate ($KMNO_4$) was added to each sample. Then prepared samples were run through machine after sample blank to analyse Hg and As metal content and results were monitored on screen attached with machine. Tubes were washed with HCL after discarding analysed sample to avoid contamination. Final results were then calculated by subtracting blank's reading from evaluated readings and multiplied them with dilution factor of 50 and then 10. After that, all the readings were converted to ppm from ppb by dividing them with 1000.

2.5 Statistical Analysis

Data was analysed after getting results using Microsoft Excel 2016. First, Basic Statistics such as minimum (Min), maximum (Max) and mean were computed on final readings. The results were presented in graphical form (bar graphs) to extract meaningful results.

2.6 Human Health Risk Assessment

2.6.1 The Translocation Factor

The translocation factor (TLF) is the ratio of HM concentration in wheat grains and shoot; it is utilized to calculate the PTEs mobility within a plant especially from vegetative (shoot) to reproductive/edible parts (grain) (Vystavna et al. 2015).

The following equation was used to estimate the translocation factor

$$\mathrm{TLF} = \frac{\text{PTEs concentration in grain}}{\text{PTEs concentration in shoot}}$$

2.6.2 Estimation of Daily Intake

The daily intake of HM estimation depends upon the content of metals in the respective food material and the rate of consumption of that food. PTEs concentration measured in wheat grains were utilized for the calculation of daily intake exposure (DIE) as a result of wheat consumption. According to the methodology of US-EPA (2011), DIE in the current study was computed by the usage of following

$$\mathrm{DIE} = \frac{\mathrm{Cgrain} \times \mathrm{DIgrain} \times \mathrm{K} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT}}$$

The factors of the abovementioned equation are explained in Table 2.6.2.

2.6.3 Non-Carcinogenic Risk

Non-carcinogenic health risk is expressed in term of hazard quotient (HQ) instead of probability. The hazard quotient (HQ) compares DIE to the reference dose (RfD), a methodology developed by US environment protection agency (US-EPA 2010) mentioned in Table 2.6.3. Both the hazard quotient (HQ) and hazard index (HI) were calculated to assess the chronic non-cancer risks of PTEs via dietary exposure. The HQs values of PTEs were predicted using the following equation

$$HQ = DIE/RfD$$

The combine risk of various PTEs is denoted as hazard index (HI) which can be calculated by following equation (US-EPA 2006).

$$HI = HQ1 + HQ2 + HQ3 \ldots\ldots\ldots\ldots\ldots HQ\ n$$

Where HQ n represents the hazard quotient (HQ PTE) from 1 to nth HMs. The HI value > 1 is associated with negative health effects.

2.6.4 Cancer Risk

Carcinogenic health hazard expressed as the incremental probability of cancer development in an individual as a consequence of exposure to potential carcinogen during lifetime. Carcinogenic risks were determined using following formula (Eq. 6):

$$CR = DIE \times SFC$$

where, SFC represents cancer slope factor.

Table 2.6.2. Parameters for Human Health Risk Assessment.

Sr No.	Factor	Description	Unit	Value		References
				Adult	Child	
1	Cgrain	Heavy Metal Concentrations in grain	mg kg^{-1}	-	-	Present Study
2	K	Conversion Factor	-	0.085	0.085	Yousaf et al. 2016b
3	DI	Daily Intake of Food Crops	kg day^{-1}	0.345	0.232	Wang et al. 2012; Liu et al. 2013
4	BW	Average Body Weight	Kg	70	32.7	US-EPA 2011
5	ED	Exposure duration	Years	30	6	US-EPA 2011, 2013; Abbasi et al. 2013
6	EF	Exposure frequency	days $year^{-1}$	365	365	US-EPA 2011, 2013
7	AT	Average time	Days	365×ED	365×ED	US-EPA 2011

Table 2.6.3. Reference Doses and Cancer Slope Factor of Heavy Metals for Human Health Risk Assessment

Heavy Metals	RfD (mg/kg)	Reference	Cancer Slope Factor (SFC)	Reference
Pb	5.00E-02	USEPA 2010; Abbas et al., 2017	8.50E-03	Ahmed et al.,2017
Cr	1.5E-0	Ghanati et al., 2019	5.00E-01	Zeng et al., 2015 (CALEPA)
As	3.00E-04	Ghanati et al., 2019	1.50E+00	Ahmed et al., 2017
Hg	3.00E-04	Ghanati et al., 2019		
Cd	1.0E-03	Ghanati et al., 2019	0.38	

Chapter 3

RESULTS AND DISCUSSION

The concentrations of different heavy metals i.e. Pb, Cr, Cd, As and Hg in grains and straws of the wheat are discussed below.

3.1 Heavy Metal Analysis in Wheat

3.1.1 Lead (Pb)

Lead is a bluish-gray metal which is naturally present in the earth's crust in a small amount. Other factors also contribute to the release of high concentrations of lead in the environment, anthropogenic activities such as fossil fuels burning, manufacturing, and mining. Lead has many different agricultural, industrial, and domestic applications. It is used in the production of lead-acid batteries, metal products (solder and pipes), ammunitions and devices to shield X-rays (Gabby, 2003; Gabby, 2006).

In recent years, the industrial use of lead has been remarkably reduced from paints and ceramic products, caulking, and pipe solder (Tchounwou et al., 2014). Inhalation of lead-contaminated dust particles or aerosols, and ingestion of lead-contaminated food, water, and paints may result in lead related diseases. Acute exposure to lead can cause brain damage, kidney damage, and gastrointestinal diseases, while chronic exposure may cause adverse effects on the blood, central nervous system, blood pressure, kidneys, and vitamin D metabolism (Tchounwou et al., 2014). The concentration of Pb in grains and straws of the wheat plant irrigated with different sources of water is shown in Table 3.1.

Industrial Effluent

Total of 16 samples (8 straw samples and 8 grain samples) with replicates analysed for the concentration of Pb in wheat out which 53 % of samples showed high concentration of Pb above permissible limit (0.30 mg/kg) recommended by FAO/WHO (Khan et al., 2018). The concentrations of Pb in grain of wheat samples irrigated with industrial effluent were in the range of (0.04-6.48 mg/kg) and in straw (0.0-6.7 mg/kg). The maximum concentration in grain collected from the area of Khayali Bypass (6.4 mg/kg) and in straw from Naroki (4.665 mg/kg).

The maximum mean level of Pb was found in the samples of grain collected from Khayali Bypass (6.4 mg/kg) followed by samples of straw from Naroki (4.665 mg/kg) and Garjakh (1.92 mg/kg). The lowest mean value was found in samples of straw collected from Shah kot (0.0 mg/kg). The concentration of Pb in all the samples of wheat irrigated with industrial effluent were above the maximum permissible limit except samples of Shahkot. Akhtar et al. (2014) conducted a study in four major peri-urban agricultural area of Pakistan including; Gujranawala, Lahore, Kasur and Multan. From Gujranwala total of 30 plant samples (vegetables/crops) were collected and analyzed for their metal contents. This study closely related to the current study, which also revealed that Pb was above the permissible limit in almost all samples of wheat irrigated with industrial effluent which were collected from Gujranwala. It is concluded that industrial effluent contains high level of Pb concentration which causes contamination in crops and vegetables.

Fytianos et al. (2001) conducted a study in which he compared the heavy metal (Pb, Cd, Ni, Cu, Mn and Zn) contents in 60 soil and vegetable samples grown in industrialised and rural areas. He recorded no differences in the heavy metal content of the vegetables grown in green houses and in urban areas, yet but significant differences were observed in plants grown in industrialised and, respectively, rural area. Study concluded that heavy metal accumulation was lower at the beginning of the irrigation period in the plants, but it would linearly increase with the rise of the population and industrialisation in Konya. Singh et al. (2010) reported that Pb, Cd, and Ni concentrations in all vegetables around the Dinapur sewage treatment plant, India, exceeded the permissible limits set by WHO/FAO. Zhuang et al. (2009) also observed Pb and Cd levels above the permissible limits in all vegetables around Dabaoshan mine, China.

Canal Water

Total of 32 samples (16 straw samples and 16 grain samples) with replicates analysed for the concentration of Pb in wheat in which 43% of samples showed high concentration of Pb above permissible limit (0.30 mg/kg). The concentrations of Pb in wheat samples of grain irrigated with canal water were in the range of 0.09-3.27 mg/kg and in straw 0.11-5.33 mg/kg. The maximum concentration in straw collected from the area of Sabzi Mandi (5.33 mg/kg) and in grains from Amli wala (3.27 mg/kg). The maximum mean level of Pb was found in the samples of straw collected from Sabzi Mandi (2.805 mg/kg) followed by samples from Amli wala (2.745 mg/kg) and Senara (2.725 mg/kg). The lowest mean value was found in samples of grain collected from Aroop Pind (0.09 mg/kg).

Municipal waste water and industrial effluent in Pakistan are drained directly into rivers, streams and irrigation canals without prior treatment (Nafees et al., 2011) and accumulate in the river, canal bed or reach to agriculture field. This is a main reason of increased level of non-essential heavy metals (Manzoor et al., 2006).

Tubewell water

Total of 42 samples (21 straw samples and 21 grain samples) with replicates analysed for the concentration of Pb in wheat out of which 48% of samples revealed high level of Pb above limit. The concentrations of Pb in straw of wheat samples irrigated with tubewell water were in the range of 0.01-1.76 mg/kg and in grain 0.03-6.67 mg/kg with the maximum concentration in straw collected from the area of Batla Pind (6.67 mg/kg) and in grains from Kot Maitala (1.76 mg/kg).

The maximum mean level of Pb was found in the samples of straw collected from Batla Pind (3.465 mg/kg) followed by samples from Kot Maitala (1.645 mg/kg) and Kot Ghulam Rasool (1.355 mg/kg). The lowest mean value was found in samples of grain collected from Maniawala (0.13 mg/kg). The concentration of Pb in all the samples of wheat irrigated with canal water were above the maximum permissible limit (0.30 mg/kg) except samples of grains from Maniawala. The descending trend generated by concentration of Pb in wheat irrigated with different sources of water was found to be industrial effluent (Straw 2.12±1.40 mg/kg)> industrial effluent (Grain 1.93±1.97 mg/kg)> canal (Straw 1.37±0.94 mg/kg)> canal (Grain 0.64±0.49 mg/kg)> tubewell (Straw 0.69±0.73 mg/kg)> tubewell (Grain 0.38±0.22 mg/kg) shown in figure 3.1.1.

In general, the wastewater irrigated food crops have higher Pb concentrations as compared to tube well water irrigated counterparts. Khan et al. (2014) found that Pb (a non-essential element) have exceeded the permissible limits of FAO/WHO in all the selected Market Food Crops including wheat (*Triticum aestivum*) of the Sialkot and Gujranwala districts. These higher Pb and Cd concentrations in the MFCs may be due to their translocation from soil (Zheng et al. 2007; Wang et al. 2012).

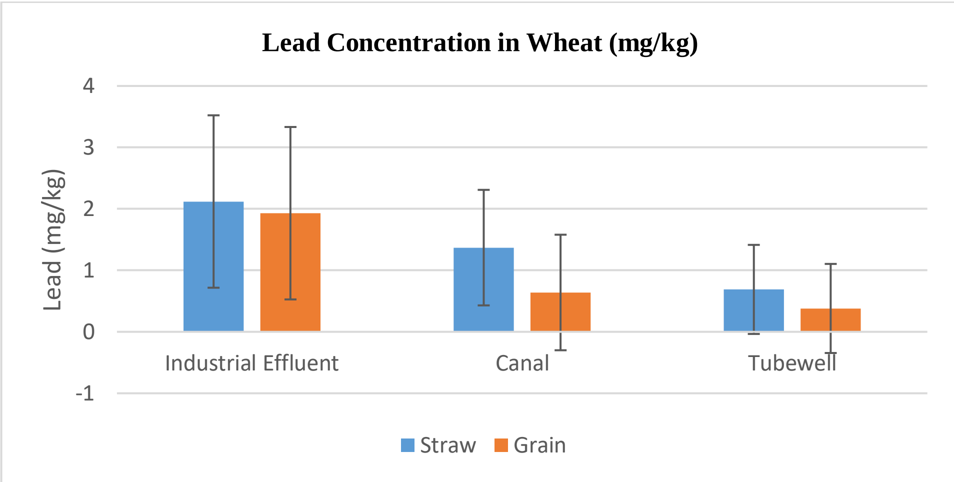

Figure. 3.1.1 Concentration of Lead in Wheat (mg/kg).

3.1.2 Chromium (Cr)

Chromium (Cr) is an element present naturally in the earth's crust, it has oxidation states (or valence states) ranging from chromium (II) to chromium (VI) (Jacobs and Testa, 2005). It is only stable in the trivalent [Cr(III)] form and occur in nature in the form of ores, such as ferrochromite. The second-most stable state form is hexavalent [Cr(VI)] (Patlollabet al., 2009). Due to natural and anthropogenic sources, chromium enters into various environmental mediums (air, water, and soil) with the major release coming from industrial establishments. The largest chromium contributed industries include stainless steel welding, metal processing, tannery facilities, chromate production, ferrochrome and chrome pigment production. The major release of Cr in the environment has been linked to air and wastewater release mainly from metallurgical, refractory, and chemical industries. Exavalent chromium [Cr(VI)] is classified as human carcinogen by several regulatory and non-regulatory agencies (USEPA,1992).

Chromium compounds are commercially used in industrial welding, chrome plating, dyes and pigments, leather tanning and wood preservation. Chromium is also used in cooking systems and boilers as anticorrosive. However, Cr has two types of exposures: one is occupational exposure it causes high risk in industrial workers those are occupationally exposed to Cr(VI). Non-occupational exposure is due to ingestion of Cr contaminated food and water whereas occupational exposure occurs only via inhalation (Tchounwou et al.,2014).

Industrial Effluent

Out of total samples of wheat irrigated with industrial effluent were analysed and revealed that about 40% of samples were above permissible limit (2.30 mg/kg) recommended by FAO/WHO (Khan et al., 2018). Chromium concentrations in straws of wheat plants irrigated with industrial effluent were found in the range (1.04-5.42 mg/kg) and in grains (0.25-3.26 mg/kg). Among straw and grain, maximum Cr concentration was found in straw from the Pindi Bypass area (5.42 mg/kg), whereas in grain, Cr was highest in samples from Naroki (3.26 mg/kg, respectively). The high levels in the green parts of the plants indicated the tendency of this metal for bioaccumulation there (Al-Othman et al., 2016).

Among the locations, the mean level of Cr was found to be maximum in Pindi Bypass (5.25 mg/kg), followed by Mandi (4.84 mg/ kg). The lowest mean level was found in Shah Kot (0.27 mg/kg). Furthermore, the level of Cr in most of the samples was found to be below the maximum permissible level of Cr in wheat (2.30 mg/kg) recommended by FAO/WHO (Khan et al., 2018). Similarly, Mapandaa et al., (2005) reported high metals in vegetable samples irrigated with wastewater.

Canal Water

Out of 32 total samples of wheat irrigated with canal showed that about 14% of samples were above permissible limit (2.30 mg/kg) recommended by FAO/WHO (Khan et al., 2018). The concentrations of Cr in straws of wheat samples irrigated with canal water were in the range of (0.25-5.82 mg/kg) and in grains (0.01-1.87 mg/kg). The maximum concentration in straw collected from the area of Rahowali (5.82 mg/kg) and also high concentration was found in grains of Rahowali (1.87 mg/kg). The maximum mean level of Cr was found in the samples of straw collected from Rahowali (3.74 mg/kg) followed by samples from Wapda Town (2.73

mg/kg). The lowest mean value was found in samples of grain collected from Chakoki (0.08 mg/kg).

Tubewell water

Total of 42 samples of wheat irrigated with tubewell analyzed and about 14% of samples showed values above permissible limit (2.30 mg/kg) recommended by FAO/WHO (Khan et al., 2018). The concentrations of Cr in straw of wheat samples irrigated with tubewell water were in the range of (0.06-5.88 mg/kg) and in grain (0.0-4.21 mg/kg). The maximum concentration in straw collected from the area of Batiuyan wala (5.9 mg/kg) and in grains from Morh Aimana abad (3.62 mg/kg).

The maximum mean level of Cr was found in the samples of straw collected from Batiuyan wala (2.99 mg/kg) followed by samples from Lodhay wala (2.96 mg/kg). The lowest mean value was found in samples of grain collected from Muslim Chowk (0.00 mg/kg). The descending trend generated by concentration of Cr in wheat irrigated with different sources of water was found to be industrial effluent (Straw 2.87±1.57 mg/kg)> canal (Straw 2.11±0.69 mg/kg)> tubewell (Straw 1.7±0.94 mg/kg)> industrial effluent (Grain 1.18±1.15 mg/kg)> tubewell (Grain 0.69±0.71mg/kg)>canal (Grain 0.57±0.34 mg/kg) shown in figure 3.1.2.

The concentration of chromium was quite low in all the samples when compared with the upper permissible limits of 8 mg/kg as identified by Soltanpour (2010) and Mushtaq & Khalid (1985). Some studies conducted on heavy metals accumulation revealed that wheat plant can uptake Cr, Cd and Ni (Herawati et al., 2000; Khan, 2001). Sutapa and Bhattacharyya (2008) reported wheat grain accumulate heavy metals, especially non-essential elements and this accumulation can be discouraged by lime treatment. In comparison of Cr, wheat grain accumulates less Cd and Ni (Bermudez et al., 2011). Another study (Hussain and Khan, 2011) revealed that Cr, Ni and Cd levels were 1.26, 2.03 and 0.02 mg/kg, respectively in soil while levels of heavy metals in wheat seed was 0.01, 0.09 and 0.00 mg/kg, respectively.

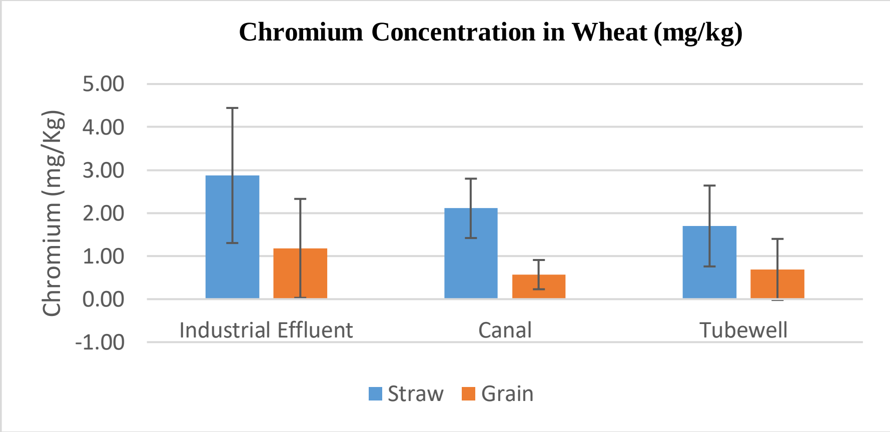

Figure.3.1.2 Concentration of Chromium in Wheat (mg/kg).

Organic pollutant and heavy metals contaminations are common in sewage. So, organic pollutants in wastewater and irrigation areas need to be investigated as well as heavy metals (Akhtar et al.,2014).

3.1.3 Mercury (Hg)

Mercury is a heavy metal which is found in nature in three forms (elemental, inorganic, and organic) and it is toxic in nature in every from (Clarkson et al., 2003). Elemental mercury exists as a liquid at room temperature and also it has a high vapor pressure and is released into the environment as mercury vapor. It also exists with oxidation states of +1 (mercurous) or +2 (mercuric) as a cation (Guzzi and La Porta, 2008). The most frequently encountered compound of Hg in the environment is methylmercury which forms as a result of the methylation of inorganic (mercuric) forms of mercury by microorganisms found in soil and water (Dopp et al, 2004).

Mercury is a widespread environmental pollutant and toxicant which causes a wide range of adverse health effects and severe alterations in the body tissues (Bhan and Sarkar, 2005). Various chemical forms of mercury in the environment are toxic to both humans and animals. Mercury is used in several industries and in processes of industries including the production of caustic soda, in nuclear reactors, used as antifungal agents for wood processing, as a solvent for reactive and precious metal, utilized in the electrical industry (switches, thermostats, batteries), dentistry (dental amalgams), and as a preservative of pharmaceutical products (Tchounwou et al., 2003).

Industrial Effluent

16 samples of wheat collected from Gujranwala irrigated with industrial effluent were analysed for the concentration of Hg showed that 28% of samples were above permissible limit (0.10 mg/kg) recommended by FAO/WHO (2016). The concentrations of Hg in wheat plants irrigated with industrial effluent were found in the range (0.0-2.21 mg/kg) and in grains (0.0-3.96 mg/kg). Among straw and grain, maximum Hg concentration was found in grains from the Nawab Chowk (3.96 mg/kg), whereas in straw, Hg was highest in samples from Garjakh (2.21 mg/kg, respectively). Among the locations, the mean level of Hg was found to be maximum in Nawab Chowk (1.995 mg/kg), followed by Garjakh (1.135 mg/ kg). The lowest mean level was found to be 0.00 mg/kg in most of the samples**.** Li and Ma et al. (2006;2010) also reported higher level of heavy metals in wheat grown irrigated with wastewater as compared to those at the CWI site.

Canal Water

Out of 32 samples of wheat irrigated with canal water about 9% of samples were above permissible limit (0.10 mg/kg) given by FAO/WHO (2016). The concentrations of Hg in straw of wheat samples irrigated with canal water were in the range of (0.0-3.56 mg/kg) and in grains (0.0-1.76 mg/kg). The maximum concentration in straw collected from the area of Wapda Town (3.56 mg/kg) and also high concentration was found in grains of Wapda Town (1.76 mg/kg). The maximum mean level of Hg was found in the samples of straw collected from Wapda Town (3.51 mg/kg) followed by samples from Amliwala (0.21 mg/kg). The lowest mean value was found in samples was 0.00 mg/kg.

Tubewell Water

14% of samples out of total showed high concentration of Hg above permissible limit (0.10 mg/kg) given by FAO/WHO (2016). The concentrations of Hg in wheat samples irrigated with tubewell water were in the range of (0.0-1.85 mg/kg) and in grains (0.0-2.22 mg/kg) with the maximum concentration in grains collected from the area of Ludhay Wala (2.22 mg/kg) and in straws from Batla Pind (0.96 mg/kg). The maximum mean level of Hg was found in the samples of grains collected from Ludhay Wala (1.12 mg/kg) followed by samples from

Kot Ratta (1.45 mg/kg). The lowest mean value was found in most of the samples were 0.00 mg/kg. The descending trend generated by concentration of Hg in wheat irrigated with different sources of water was found to be industrial effluent (Straw 0.32±0.46 mg/kg)> industrial effluent (Grain 0.32±0.68 mg/kg)> canal (Straw 0.23±0.87 mg/kg)> tubewell (Straw 0.14±0.26 mg/kg)> canal (Grain 0.1±0.37 mg/kg) > tubewell (Grain 0.09±0.39 mg/kg) shown in figure 3.1.3.

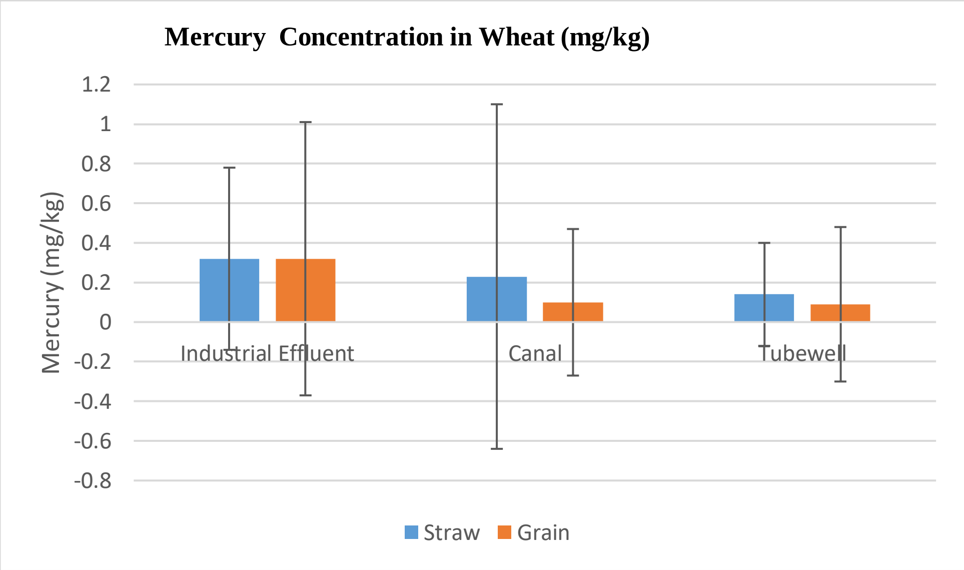

Figure 3.1.3 Concentration of Mercury in Wheat (mg/kg).

A study conducted by Okoronkwo et al. (2005) proved that high level of metal contamination becomes a source of food chain and ultimately cause high exposure of toxic elements in human and animal via food chain, ingestion of windblown dust or direct ingestion. The high quantity of heavy metals can badly affect the growth and production of plants or crops, if heavy metals accumulated in the soil which causes their translocation to plants (Moftah et al., 2000).

3.1.4 Arsenic (As)

Arsenic is a universal element that is detected even at low concentrations if present in any environmental medium. Natural phenomena such as volcanic eruptions and soil erosion, and anthropogenic activities may lead to cause environmental pollution of arsenic (Tchounwou et al, 2014). Several compounds that contain arsenic produced industrially, and also these compounds have been used to manufacture products with agricultural applications such as insecticides, herbicides, fungicides, algicides, wood preservatives, and dye-stuffs. They have also been used in veterinary medicine for the eradication of tapeworms in sheep and cattle (Tchounwou et al, 2014).

For at least a century, arsenic compounds have also been widely used in the medical field in the treatment of syphilis, yaws, amoebic dysentery, and trypanosomaiasis (Centeno et al., 2005). Several million people are exposed to arsenic chronically throughout the world, especially in countries like Bangladesh, India, Chile, Uruguay, Mexico, Taiwan, where the ground water is contaminated with high concentrations of arsenic. The largest source of exposure for most of individuals is diet because a person intakes an average of about 50 µg per day. Intake from other sources such as air, water and soil are usually much smaller, but exposure from all medium may become significant in areas of high arsenic contamination. High levels of arsenic contamination is of great concern because arsenic exposure can cause a number of human health effects. Several epidemiological studies have reported a strong association between arsenic exposure and increased risks of both carcinogenic and systemic health effects (Tchounwou et al., 2003).

Industrial Effluent

16 samples of wheat collected from Gujranwala irrigated with industrial effluent were analysed for the concentration of As showed that 31% of samples were above permissible limit (0.10 mg/kg) recommended by FAO/WHO (2016). The concentrations of arsenic (As) in different parts of the wheat (straw and grain) that was irrigated with industrial effluent were in the range 0.00– 1.09 mg/kg. The highest concentrations were found in samples of wheat straw and grain collected from PindiBypass (0.24 and 0.19 mg/kg). The considerable level of As in aerial parts suggested that the wheat plant is a strong bioaccumulator (Al Othman et al.,2016). The high metal concentrations disrupt the outer membranes and collapse organelles in the cell (Stresty et al., 1999). It also acts as mutagenic substance (Sharmaa et al.,2007) causing the disorder in the physiological functions, i.e., photosynthesis (Van et al., 1990). The pattern of accumulation of As in the various parts of the plant was straw> grain. The study of the distribution of As revealed that the maximum mean level was found in Naroki (1.06 mg/ kg),

followed by Pindi Bypass (0.24 mg/kg) and Khayali Bypass (0.17 mg/kg). Meanwhile, the lowest mean level was observed (0.00 mg/kg) in most of the samples.

Canal Water

Out of 32 samples of wheat irrigated with canal water about 17% of samples were above permissible limit (0.10 mg/kg) given by FAO/WHO (2016). The concentrations of arsenic (As) in straw of wheat irrigated with canal water were in the range of 0.0-3.04 mg/kg and in grains 0.0-1.03 mg/kg. The highest concentrations were found in samples of wheat straw collected from Kot Mirchan (3.04 mg/ kg) and in samples of grain collected from Kot Ratta (1.03 mg/kg). The pattern of accumulation of As in the various parts of the wheat was straw> grain. The study of the distribution of As revealed that the maximum mean level was found in Kot Mirchan (1.53 mg/ kg) and the lowest mean level was observed (0.00 mg/kg) in most of the analysed samples of wheat.

Tubewell water

Out of 42 samples of wheat irrigated with tubewell collected 40% of samples showed high concentration of Hg above permissible limit (0.10 mg/kg) given by FAO/WHO (2016). Arsenic content in the tested straw samples of wheat irrigated with tubewell water occurred in the range (0.0-4.73) mg/kg and in grains (0.0-2.58) mg/kg. The highest level was found in plant straw samples collected from Batiuyan wala (4.73 mg/kg) and also in grains from samples of Batiuyan wala (2.58 mg/kg). The lowest level was observed (0.00 mg/kg) in most of the samples of wheat. The general behavior for the accumulation of As in wheat was straw>grain. The highest mean level of As was found in wheat from Batiuyan wala (4.73 mg/kg) and Lodhay wala areas (3.345 mg/kg), followed by Aimanabad (1.01 mg/kg). The lowest mean level was observed (0.00 mg/kg) in most of the samples of wheat. In general, however, the level of As in most of the samples were below the maximum permissible level (0.10 mg/ kg) recommended by FAO/WHO (2016) except some samples of wheat.

The descending trend generated by concentration of As in wheat irrigated with different sources of water was found to be tubewell (Straw 0.75±1.27 mg/kg)> canal (Straw 0.27±0.44 mg/kg)> tubewell (Grain 0.24±0.60 mg/kg) > industrial effluent (Grain 0.17±0.37 mg/kg)>

industrial effluent (Straw 0.04±0.09 mg/kg)> canal (Grain 0.04±0.13mg/kg) shown in figure 3.1.4.

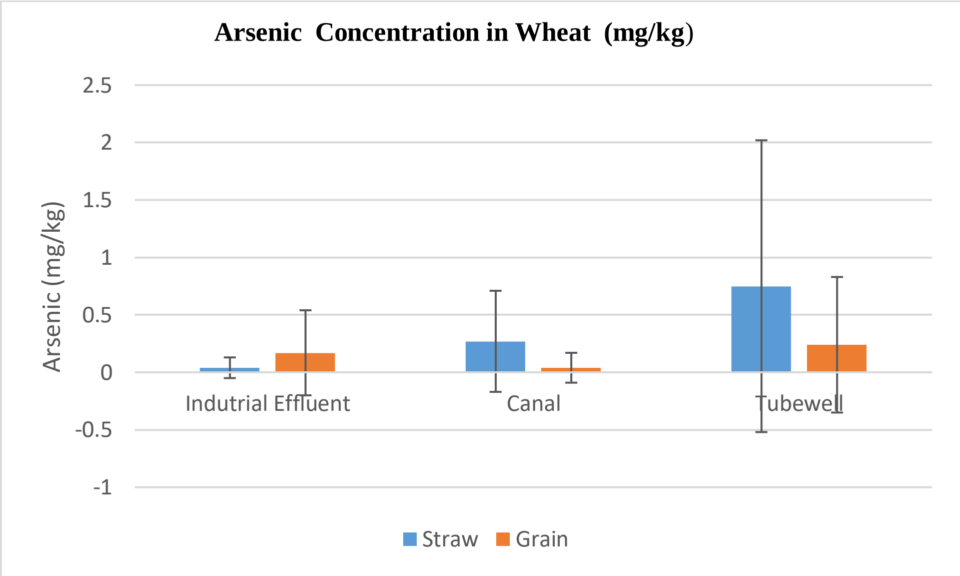

Figure 3.1.4 Concentration of Arsenic in Wheat (mg/kg).

Baig et al. (2011) investigated the level of As contamination in groundwater and in common grain crops. Results showed that the levels of total arsenic (TAs) in all test grain samples (TGS), irrigated on soil irrigated with tube well (SIT) of Faiz Ganj, Thari Mirwah and Gambat, were found to be higher than in those grown on soil irrigated with canal water (SIC). High translocation of As to grains from tube well water and SIT was predicted in this study. Similar trend was also observed in Bangladesh (Islam et al., 2007). This study is related to current study in which high As was found in tubewell irrigated wheat crops as compared to canal and wastewater irrigated wheat samples.

3.1.5 Cadmium (Cd)

Cadmium is broadly distributed in the earth's crust at an average concentration of about 0.1 mg/kg with significant environmental and occupational concern. Cadmium has significant role in various industrial activities include the production of alloys, pigments, and batteries (Tchounwou et al., 2014). Although in recent years, the cadmium's use in batteries has grown.

But in developed countries, its commercial use has declined in response to environmental concerns.

Cadmium is exposure through routes via inhalation or cigarette smoke, and ingestion of food. In certain foods, cadmium is also found in trace amounts such as leafy vegetables, potatoes, grains and seeds, liver and kidney, and crustaceans and mollusks (Satarug et al., 2003). Due to frequent use of cadmium in industries, the environmental contamination and human exposure to cadmium have intensely increased during the past century (Elinder et al.,1996). Acute effects of cadmium through ingestion can also cause gastrointestinal tract erosion, pulmonary, hepatic or renal injury and coma, depending on the route of poisoning (Tchounwou et al., 2014).

Industrial Effluent

The level of Cd in different wheat irrigated with industrial effluent ranged from a minimum of 0.19 mg/kg to a maximum of 3.77 mg/kg (Table 2). The highest concentration (3.77 mg/kg) was found in wheat straws from the Shahkot area and also (2.01 mg/kg) in plant grains from the Shahkot area. It was also observed that the general pattern for the distribution of Cd in different parts of the plant was straw>grain. The distribution of Cd revealed that the highest mean level was also found in samples from Shahkot (3.77 mg/kg) followed by samples from the Khayali Bypass area (3.56 mg/kg). The lowest mean level was observed in samples of grains obtained from the Nawab Chowk (0.19 mg/kg).

The concentration of Cd in all the samples of wheat were above the permissible limit of Cd (0.20 mg/kg) except samples obtained from Nawab chowk. In 100 % plants samples collected from Gujranwala that were irrigated with industrial effluent showed higher Cd concentration reported by Akhtar et al. (2014), indicated that high level of Cd is also present in industrial effluent above permissible limit also causes contamination in plants and crops. This study is related to current study which shows Cd in almost all samples of wheat irrigated with industrial effluent collected from peri urban areas of Gujranwala. In another study, Khan et al. (2019) reported that the mean concentrations of Cd, Cu and Cr were increased by sewage water irrigation in wheat grains that were irrigated by five different treatments of water. Contamination of Cd in food crops and its effects on human health have also been broadly reported (Yang et al., 2018).

Canal Water

The concentrations of Cd in wheat samples irrigated with canal water were in the range of (0.01– 2.35 mg/kg) with the maximum concentration in straw collected from the area of Rahowali (2.35 mg/kg) and also high concentration was found in grains of 1km from Aroop pind (1.56 mg/kg). The maximum mean level of Cd was found in the samples of straw collected from Rahowali (2.35 mg/kg) followed by samples from 1km from Aroop pind (2.18 mg/kg). The lowest mean value was found in samples of grain collected from Najaf Colony (0.01 mg/kg). The concentration of Cd in most of the samples of wheat irrigated with canal water were below the permissible limit of Cd (0.20 mg/kg) except some samples that shows concentration below limits recommended by FAO/WHO guidelines.

Tubewell water

The concentrations of Cd in wheat samples irrigated with tubewell water were in the range of (0.02 – 0.43 mg/kg) with the maximum concentration in straw collected from the area of Kot Maitala (0.43 mg/kg) and also in grains from Kot Maitala (0.24 mg/kg). The maximum mean level of Cd was found in the samples of straw collected from Kot Maitala (0.43 mg/kg) followed by samples from Kotli (0.39 mg/kg) and Aimanabad (0.37 mg/kg). The lowest mean value was found in samples of grain collected from Dera Sultan and Taragri (0.02 mg/kg). According to Hussain et al. (2010) Cd concentration was comparatively low in wheat seed irrigated with tubewell water than shoot and root. In another study, lower Cd concentration in wheat grains was also observed by Adriano (2011). The concentration of Cd in almost all of the samples of wheat irrigated with tubewell water were below the maximum permissible limit (2.30 mg/kg) recommended by FAO/WHO guidelines except some samples which show high concentration of Cd above permissible limit. In one of the study from district Sargodha, the highest concentrations of Cd in the soil was found to be 6.74 mg/Kg and it may cause the possible risk of Cd entering into higher food chain which was reflected by the Cd accumulation by forage in the range of 1.14 to 4.20 mg/kg (Khan et al., 2011).

The descending trend generated by concentration of Cd in wheat irrigated with different sources of water was found to be industrial effluent (Grain 1.79±0.76mg/kg)> industrial effluent (Straw 1.04±1.36 mg/kg)> canal (Grain 0.77±0.55 mg/kg) > canal (Straw 0.32±0.83

mg/kg) > tubewell (Grain 0.19±0.07mg/kg) > tubewell (Straw 0.09±0.11 mg/kg) shown in figure 3.1.5.

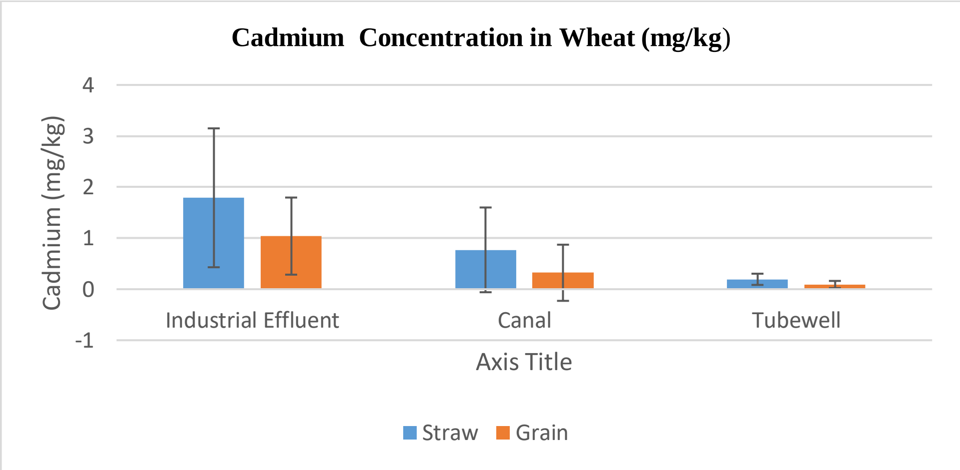

Figure 3.1.5. Concentration of Cadmium in Wheat (mg/kg).

Table.3.1 Permissible limits of heavy metals in wheat (mg/kg).

Sr No.	HEAVY METALS	FAO/WHO 2001*	FAO/WHO 2016** (Fooditems)
1.	Cd	0.2	-
2.	Pb	0.3	-
3.	Cr	2.3	-
4.	As	Not defined*	0.10
5.	Hg	-	0.10

*Khan et al., 2019.
** Hussain et al, 2019.

Table.3.2 Heavy Metals Content in Wheat of Peri-Urban Areas of Gujranwala (mgkg^{-1}).

Sr. No	Categories	Lead (Pb)		Chromium (Cr)		Mercury (Hg)		Arsenic (As)		Cadmium (Cd)	
		Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
1	**Industrial effluent**										
	Grain	0.04-6.48	1.93±1.97	0.25-3.26	1.18±1.15	0.0-3.96	0.32±0.68	0.0-1.09	0.17±0.37	0.2-3.77	1.79±0.76
	Straw	0.0-6.7	2.12±1.40	1.04-5.42	2.87±1.57	0.0-2.21	0.32±0.46	0.0-0.24	0.04±0.09	0.19-2.01	1.04±1.36
2	**Canal Irrigation**										
	Grain	0.09-3.27	0.64±0.49	0.01-1.87	0.57±0.34	0..0-1.76	0.1±0.37	0.0-1.03	0.04±0.13	0.11-2.35	0.77±0.55
	Straw	0.11-5.33	1.37±0.94	0.25-5.82	2.11±0.69	0.0-3.56	0.23±0.87	0.0-3.04	0.27±0.44	0.01-1.56	0.32±0.83
3	**Tubewell Irrigation**										
	Grain	0.01-1.76	0.38±0.22	0.0-4.21	0.69±0.71	0.0-2.22	0.09±0.39	0.0-2.58	0.24±0.60	0.06-0.43	0.19±0.07
	Straw	0.03-6.67	0.69±0.73	0.06-5.88	1.7±0.94	0.0-1.85	0.14±0.26	0.0-4.73	0.75±1.27	0.02-0.24	0.09±0.11

3.2 Human Health Risk Assessment

3.2.1 Translocation Factor (TLF)

Higher concentration of HMs taken up by plants through roots from growing media, translocate and hoard them in aerial parts (McGrath et al. 2001). The translocation factor (TLF) from shoot to edible portion (grain) of wheat is depicted in Table 3.2.1. The translocation factor reveals immense diversity in wheat irrigated with industrial effluent, canal, and tubewell with pattern of Pb > Hg >Cd > Cr >As.

Table. 3.2.1. Translocation factor of HMs in wheat shoot-grain

Site	**Heavy Metals**				
	Pb	**Cr**	**Hg**	**As**	**Cd**
Industrial Effluent	1.66E+00	4.41E-01	0.00E+00	9.64E-02	6.19E-01
Canal	5.93E-01	2.96E-01	8.16E-02	4.24E-01	3.50E-01
Tubewell	7.38E-01	5.07E-01	5.47E-01	1.64E-01	4.51E-01

3.2.2 Estimated Dietary Exposure (DIE)

Assessment of human health risk of HMs and possible levels of exposure associated by tracking the pathways of contaminant exposure in a target living organism is vital (Liu et al., 2013). Although, Human are highly exposed to HMs pollutants through number of sources such as soil, air, water, and food (Caussy et al., 2003). However, human vulnerability to these HMs through ingestion via polluted food has been considered as principal pathway of exposure (Yousaf et al., 2016c). The below discussion pointed out exposure of HMs pollutants by staple food (wheat) utilization in peri-urban areas of Gujranwala district.

A significant volume of HMs ingested by the locality where wheat grown in that area irrigated with contaminated water with non-point and point sources of metal pollutants. The dietary daily intake exposure (DIE) of HMs to children and adults linked with the wheat consumption (mg kg^{-1} day^{-1}) are evaluated at all studied sites and presented in figure 3.2.2. As stated earlier, the wheat grown in the peri-urban areas of Gujranwala consumed as a staple diet by the native communities can bring about crucial health problems. The assessment of DIE was carried out by using the mean concentrations of these HMs in wheat grains. There is substantial difference of DIE (figure 3.2.2) values in all studied regions among children and adults with the consumption of wheat.

Pb, Cr and Cd have highest DIE values in wheat consumed irrigated with industrial effluent in adults whereas in children, Pb show highest DIE value. The highest values for DIE was appraised for Pb in wheat irrigated with industrial effluent in both adults and children. The lowest values of DIE were evaluated for As in wheat irrigated with canal water for both adults and children. However, in current study computed values of DIE for all the HMs were much less than unity (< 1); the safe limits of HMs in food for human ingestion. For both children and adults, the DIE via wheat consumption was much less than RfD standard set by US-EPA (Abbas et al., 2017; Ghanati et al., 2019). The comparison of DIE evaluated results with those described by Khan et al. (2013) and Yousaf et al. (2016c) revealed similar trends, which were consistent with for all HMs (< 1). The DIE values of the current study predicted that the ingestion of wheat as a staple food for local communities is safe for individual HMs. But in near future there might be severe human health hazards with the ingestion of contaminated wheat especially irrigated with industrial effluent in both adults and children.

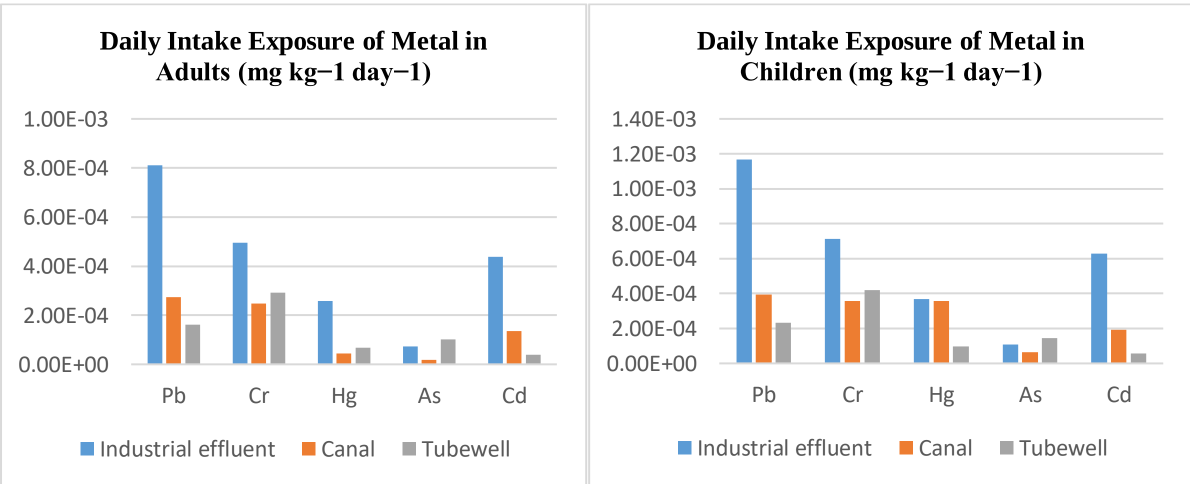

Figure 3.2.2 Daily Intake Exposure (DIE) of HMs in Adults and Children (mg kg^{-1} day^{-1}).

3.2.3 Predicted Human Health Risk of Heavy Metals

Heavy metals like Pb, Cd, Cr, As and Hg concentration in diet above safe limits causes toxicological problems in humans, and are carcinogenic (WHO, 2011). The method used to assess human exposure to the metallic pollutants and health risk assessment for the residents of Gujranwala is US-EPA (2010, 2011). The consumption of highly contaminated wheat may possess health hazards (cancerous and non-cancerous) presented in Table 3.2.3 and 3.2.4. The main focus of present study is the assessment of non-carcinogenic risk of heavy metals in adults and children as a result of consumption of wheat as a staple food in Gujranwala. The reference dose (RfD) in wheat was used to anticipate the human health risks of heavy metals via dietary exposure US-EPA (Abbas et al., 2017; Ghanati et al., 2019).

Non-Carcinogenic Risk

The chronic non-carcinogenic risk associated with wheat consumption is illustrated for children and adults in Table 3.2.3, which clearly exhibits higher level of potential risk from Hg and Cd as compared to Pb, As and Cr. The non-cancerous risk through dietary uptake show same pattern Hg >Cd >As > Pb> Cr in wheat irrigated with industrial effluent, canal and tube well for both children and adults. Overall, noncancerous hazard for children and adult was trending in order: children (Industrial effluent) > adults (Industrial effluent) > children (canal) > adult (canal) > children (Tubewell) > adult (Tubewell). The noncarcinogenic hazard results of current study are comparable with the study of Abbas et al. (2017) that was performed for the consumption of wheat in Faisalabad, Pakistan. But in this study the estimated risk levels of Pb is higher than previous study. Khan et al. (2019) reported in a study conducted in Sargodha, very high risk level of Cd in wheat irrigated with groundwater as well as wastewater than estimated in current study.

In another study, Farahat et al. (2017) concluded that HRI value of Pb and Cd in wheat irrigated with wastewater was above the standard value of 1 which poses health risk for both adults and child as compared to wheat irrigated with freshwater. When compared with present study, HRI values for Pb and Cd are below 1 according to environmental quality standards (US-EPA 2011). The cumulative non-cancerous risk of HMs is referred as hazard index (HI) was also estimated at different sites for wheat consumption. HI at all the studied sites was lower than the standard value (1) set by US-EPA (2011). For all the studied HMs, the present non-carcinogenic hazard called as target hazard quotient (HQ) and the (HI) hazard index both are within safe range so currently the consumption of wheat produced from these sites is harmless except Hg in wheat which is irrigated with industrial effluent for children. However, it is necessary to check the health risk hazards with continuous monitoring associated with intake of wheat in future.

Table 3.2.3 Predicted THQ for non-carcinogenic of HMs associated with wheat consumption.

Inhabitants	**Site**	**Heavy Metals**					
		Pb	**Cr**	**Hg**	**As**	**Cd**	**Mean***
Adults	**Industrial effluent**	1.62E-02	3.31E-04	8.57E-01	2.47E-01	4.37E-01	3.12E-01
	Canal	5.48E-03	1.65E-04	1.48E-01	6.07E-02	1.35E-01	6.98E-02
	Tubewell	3.23E-03	5.84E-03	1.35E-03	2.03E-03	7.74E-04	2.65E-03
Children	**Industrial effluent**	2.34E-02	4.76E-04	1.23E+00	3.56E-01	6.29E-01	4.49E-01
	Canal	7.89E-03	2.38E-04	2.13E-01	8.73E-02	1.94E-01	1.01E-01
	Tubewell	4.65E-03	8.41E-03	1.95E-03	2.93E-03	1.11E-03	3.81E-03

*Combine health risk (HI values) of HMs

Cancer Risk

The potential source of carcinogenic health hazard likewise non-carcinogenic hazard is also the ingestion of contaminated food. Thus, the cancer risk assessment of heavy metals associated with dietary exposure is regarded as exceedingly crucial. The cancer risk of HMs for adults and children via food uptake (wheat) was also assessed and compared with US-EPA (2011, 2013) guidelines. Cancer risk by dietary exposure was calculated for Pb, Cd, As and Cr as cancer slope factor was available only for these HMs mentioned in table 2.6.3.

The Table 3.2.4 demonstrates the cancer risk of Pb, Cd, As and Cr. Overall, the level of Pb, Cd, As and Cr carcinogenic risk was observed within the safe limit (1E-06 to 1E-04) of US-EPA (2011). Our findings for Pb cancerous hazard were much lower as compared with the study of Yousaf et al. (2016c) carried out in urban, peri-urban localities of Gujranwala for vegetables. In an another study, Abbas et al. (2017) finds out the cancer risk for Pb and Ni in wheat from industrial, urban and peri-urban areas of Faisalabad in which results showed that high level of cancer risk above than safe limit. But in current study cancer risk for both adults and children were with in safe limits.

According to the investigation performed in eastern China by Zhao et al. (2014) in wheat grains, carcinogenic risk of Pb surpassed than that in the present study; there could be many reasons and may be due different exposure channels such as dermal contact, inhalation via air, consumption of water, and soil ingestion (Okorie et al. 2011; Niu et al. 2015). Although, the cancer risk of heavy metals on both adults and children are below than the threshold levels, but with constant consumption and use of contaminated wheat could be a source of cancer in local people. So, it is necessary to remediate polluted soil to in order to abstain and mitigate human health hazards of heavy metals to local communities via food chain (Chen 2007).

Table. 3.2.4 Predicted carcinogenic risks of HMs associated with wheat consumption.

Inhabitants	**Site**	**Heavy Metals**				
		Pb	**Cr**	**As**	**Cd**	**Mean**
Adults	**Industrial effluent**	6.90E-06	2.44E-04	1.11E-04	1.66E-04	1.32E-04
	Canal	2.33E-06	1.24E-04	2.73E-05	5.12E-05	5.12E-05
	Tubewell	1.37E-06	1.46E-04	1.52E-04	1.47E-05	1.47E-05
Children	**Industrial effluent**	9.94E-06	3.57E-04	1.60E-04	2.39E-04	1.92E-04
	Canal	3.35E-06	1.78E-04	3.93E-05	7.38E-05	7.36E-05
	Tubewell	1.97E-06	2.10E-04	2.19E-04	2.12E-05	1.13E-04

Chapter 4

CONCLUSION

Current study implied that the concentration of heavy metals increased with the application of wastewater. In general, the industrial effluent irrigated wheat have higher Pb concentrations as compared to canal and tube well water irrigated wheat counterparts due to their translocation from soil. Furthermore, the level of Cr in samples irrigated with industrial effluent was found to be high as compared to wheat samples irrigated with canal and tubewell water. Most of the samples showed high concentration of Cr in wheat above maximum permissible level of Cr (2.30 mg/kg) recommended by FAO/WHO (Khan et al., 2018). The level of Hg in most of the samples was found to be below the maximum permissible level of Hg in wheat (0.10 mg/kg) recommended by FAO/WHO (2016) but wheat samples irrigated with industrial effluent showed high level of Hg as compared to canal and tubewell irrigated samples**.** The concentrations of As in various samples were below the permissible limit for As in crops (0.10 mg/kg) recommended by FAO/WHO (2016) except some samples of wheat. Many health problems in humans may cause by contaminated food. Cd in wheat samples irrigated with industrial effluent showed high concentration than samples irrigated with canal and tubewell. As Cd is easily available, soluble and easily leachable. Major sources of Cd in wastewater were food products, paints, detergents, and body care products. if concentration of heavy metals were lower in crop but due to wastewater irrigation for long time can accumulate heavy metals in the edible parts of crops which can lead towards the serious health risks causing (Cancer, Hepatitis etc) via food chain.

The trend of translocation factor was Pb > Hg >Cd > Cr >As revealed that there are more chances of heavy metals to transfer from shoot to grain. DIE values were calculated for adults and children showed that high level of Pb in wheat leads to more dietry exposure in adults and children followed by Cr, Cd, Hg and As. Hazard Quotient (HQ) and Hazard Index (HI) of analysed heavy metals showed that wheat was contaminated with these heavy metals but within safe range but with continuous consumption of contaminated wheat may cause serious health problems. So, in order to cope with these problems necessary and rapid actions should be taken to scrutinize the concentration of metals and metalloids in soils and to reduce their concentration by using phytoextraction and waste water should be treated properly before its application. Cancer risk for adults and children were also detected for heavy metals in wheat was within safe limit of (1E-06 to 1E-04) of US-EPA (2011).

Findings of current study have highlighted the significance of continued predictions of heavy metals in Gujranwala, while the assessment of cancer and non-cancer human health risks of these lethal pollutants must be employed to locate the detailed health risk situation. In addition to the public sector agencies, private organizations should also play role in the awareness of health risks of PTEs to the local public. The decision makers should also consider these risk assessment studies during making environmental policies.

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