ARSENIC ASSESSMENT IN BIOLOGICAL SAMPLES-A COMPARATIVE APPROACH OF MEAT SOLD IN DIFFERENT CITIES OF PAKISTAN

By

IQRA RASHEED

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Arsenic - Analysis

Food - Analysis

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FINAL APPROVAL

It is certificate that we have read the thesis titled as **"Arsenic Assessment in Biological Samples-A Comparative Approach of Meat Sold in Different Cities of Pakistan"** submitted by Ms. Iqra Rasheed and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the M.S Degree in Environmental Sciences.

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A thesis titled as ""***Arsenic Assessment in Biological Samples-A Comparative Approach of Meat Sold in Different Cities of Pakistan***" submitted to Department of Environmental Sciences, International Islamic University, Islamabad as a partial fulfillment of requirement for the award of the degree of M.S in Environmental Sciences

DEDICATION

I dedicate this research to Almighty ALLAH, Who helped me in accomplishing this thesis and for giving me wisdom or giving me strength to overcome pressure while doing this thesis. This humble effort is also dedicated to my beloved parents who are the symbol of guidance in my life and motivated me to accomplish this research project and whose hands always rose in prayers for my success.

DECLARATION

I hereby declare that the work present in the following thesis titled as "**Arsenic Assessment in Biological Samples-A Comparative Approach of Meat Sold in Different Cities of Pakistan**" is my own effort, except where otherwise acknowledged and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.

Date 2-11-2017

Iqra

Iqra Rasheed

Table of Contents

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May Allah bless them all (Amen).

Iqra Rasheed

ABSTRACT

The arsenic present in the liver and muscles of chicken, goat and cow were determined in the present study. This study was conducted in Lahore, Gujrat, Islamabad and Peshawar during August 2016. Seventy two meat samples were collected randomly from three different points of selected cities. Samples were transported to the laboratory by keeping in separate high density polyethylene bags and kept in refrigerator at -20°C. All samples were dried at 60°C for 24 hours than grind and converted into powdered form. One grams of each dried sample was subjected to acid digestion (2mL GR (v/v) HNO_3 (grade 65%) and $HCLO_4$). For analysis Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) was used. Statistically significant results of arsenic residual in different meat samples were found by using two way ANOVA. Spatial distribution of arsenic in the liver samples of chicken, showed highest concentration in Peshawar and lowest in Gujrat and the order presented was: Peshawar > Islamabad > Lahore > Gujrat. In case of Liver samples of goat order presented was: Gujrat > Islamabad > Peshawar > Lahore. The accumulation pattern of arsenic in liver samples of cow was as follows: Lahore > Peshawar > Gujrat > Islamabad. The residual level in muscle samples of chicken was ordered as: Lahore > Gujrat > Peshawar > Islamabad, in goat order was: Peshawar > Gujrat > Lahore > Islamabad and cow muscle samples were ordered as: Peshawar > Gujrat > Lahore > Islamabad. The Human Health Risk assessment showed high level of risk due to daily intake of arsenic, although the contamination in the meat samples was under the permissible limits but daily intake of less contaminated meat can also create high level of risk. The contamination in meat samples, indicating the presence of arsenic in water, feed and in air due to used in pharmaceuticals, wood preservatives, agricultural chemicals, mining, glass making, metallurgical and in semi conductor industries. Therefore, according to international standards of arsenic in meat, these meat types from studied location are suitable for human consumption but their daily ingestion is harmful.

LIST OF ABBREVIATION

AAFC	Agriculture and Agri-Food Canada
AAS	Atomic Absorption Spectroscopy
ANOVA	Analysis of Variance
ANZFA	Australia New Zealand Food Authority
ATSDR	Agency for Toxic Substances and Disease Registry
DEFRA	Department for Environment, Food and Rural Affairs
FAO	Food Agricultural Organization
IAEA	International Atomic Energy Agency
IARC	International Agency For Research On Cancer
IATP	Institute of Agriculture and Trade Policy
ICP-MS	Inductively Coupled Plasma- Mass Spectroscopy
MCL	Maximum Contamination Level
NRC	National Research Council
NTP	National TB Control Program
USDA	United State Department of Agriculture
USEPA	United States- Environmental Protection Agency
USPHS	United State Public Health Service Home
WHO	World Health Organization

LIST OF FIGURES

LIST OF TABLES

Chapter # 1

INTRODUCTION

INTRODUCTION

Toxic metal exhibits severe toxicological effects even at low concentration when they become unnecessary and does not exhibit positive effects on living things (Mariam *et al.*, 2004). Due to rapid increase in industrialization, metals have entered into the air, water and soil frequently (Mariam *et al.*, 2004). These toxic metals stay permanently and enter into the food chain where they ultimately make their pathway into the tissues (Baykov *et al.*, 1996).

Arsenic is a ubiquitous toxic metal found in different media like earth crust, ground water and surface water (Flora *et al.*, 2005). The atomic number of arsenic is 33 and atomic mass is 74.92, chemical and physical properties lies between metal and non metal so referred to as metalloid (WHO, 2001; IARC, 2004). In the environment, different valence states of organic and inorganic arsenic exist such as: metalloid state, oxidized state, trivalent state (arsenites) and pentavalent state (arsenates) (ATSDR, 2000). Organic arsenic is less toxic arsenic as compare to inorganic because of its insolubility in human body fluids and water (ATSDR, 2000). There are several natural and anthropogenic activities which accelerate the process of arsenic discharge into the environment which becomes ultimately burn on the biological system. Natural activities include volcanic eruption, dissolution of minerals (particularly into groundwater), residues from vegetation and windblown dust. Natural sources of arsenic contributed about one third of the global flux, approximately 7900 tons in a year (WHO, 2001). Anthropogenic activities are mining, metal smelting, combustion of fossil fuels, agricultural pesticide production and use, and timber treatment with preservatives. Remobilization of historic sources, such as mine drainage water is also the source of arsenic generation. Mobilization of arsenic into drinking-water from geological deposits by drilling of tube wells on arsenic rich areas (WHO, 2010).

During the process of weathering arsenic sulfide is transformed to arsenic trioxide and then ultimately entered in the environment with dust particles or by mixing in the river, rain and groundwater (Mandal and Suzuki, 2002). It mainly absorbed on dust particles, then dispersed by winds and deposited in water and land (WHO, 2001). After entering into biogeochemical cycle, arsenic is available to the living organism and it may cause toxicity (WHO, 2001).

There are three main routes of arsenic exposure to human such as ingestion, inhalation and dermal contact. The main route of arsenic absorption in the body is through ingestion of contaminated food. It has been found that in United States, 11-14 milligrams of arsenic ingested daily through diet by an adult. When arsenic ingested by humans 60% to 90% absorbed in the gastrointestinal tract. The amount of arsenic absorbed via inhalation is not determined accurately, but is assumed to be ranged from 60% to 90% also. Generally, arsenic trioxide is present in the air and it directly deposited in the respiratory tract. Few cases of dermal absorption have been found also from occupational accidents such as from arsenic trichloride or arsenic acid splashed on worker's skin. After entrance into lungs and gastrointestinal tract, it accumulates in the other organs of the body like liver, muscles, kidney and circulatory system (ATSDR, 2000). The methylation activity of inorganic arsenic is occurred in the liver (Vaheter, 2002). In this methylation process, inorganic arsenic is biotransformed in the presence of enzymes to methylated arsenicals including MMA and DMA, these are the biomarkers of chronic arsenic exposure (Biggs *et al.,* 1997; Thomas *et al.,* 2001). After converting arsenic into less toxic form, liver transfer the arsenic into urine, through which it passes out. There are some other elimination routes of arsenic from the body such as feces, sweat, skin desquamation and absorption into nails and hair (ATSDR, 2000).

In the current time period greatest threat to human health occurs due to contamination in ground water. In most of the countries, inorganic arsenic is present in the ground water naturally (Nkansah and Ansha, 2012). Maximum Contamination Level (MCL) is the level of arsenic concentration in water placed by the United States Environmental Protection Agency (10 μg/l), under which water is not hazardous for drinking purposes (USEPA, 2001) and US EPA's maximum contamination level in drinking water is 50ppb (ATSDR, 2000). Globally millions of people and animals use to drink water which is above permissible limits. The food, humans used as meal, may grow in the arsenical soil, water and pesticide (Nriagu *et al.*, 1990). A research conducted on food cultivated in arsenic rich area, shows that this toxic metal is not exist in the food internally but displays its toxicity on the outer part of the food may be due to sprinkle of arsenical sprays or water. Many reports show the concentration of arsenic in cow's milk because of drinking arsenicated water (Nariagu, 1990). The other animals such as sheep, buffaloes and goat used as meat also drink arsenicated water and arsenic accumulates in the animal's body.

Due to intensive and long term exposure of human to arsenic, millions of people in the world have been affected by cancer even at the low doses (Ratnaike, 2003). According to IATP, arsenic contributes to other diseases also, like heart disease, diabetes and declines in intellectual function (Institute Of Agriculture and Trade Policy, 2006). Skin manifestation is the most common and initial symptom of chronic arsenic exposure in the body (Maloney, 1996).

In omnivore society, population feed on animal proteins as well as on plant proteins. For animal protein, people depend upon different animals as sources of proteins. Proteins and vitamins present in animals have high biological value as they make a healthy diet (Bastin, 2007). Chicken, mutton and beef are the major sources. Due to process of bioaccumulation, animals have more arsenic contamination as compare to the majority of plants. The expected demand of chicken will be increase by a surprising 750% in the South Asia countries and this requirement for proteins is mainly based on the growth in urban areas. Meat and meat products become very important for human beings, apart from its importance in human diet and variety of valuable nutrients present in it, some toxic substances also exist (Fathy *et al.*, 2011). Being an Islamic country, meat of only 'halal' animals is consumed in Pakistan.

The Food and Agricultural Organization (FAO) has worked a lot to predict the increase in demand of mutton and beef in the upcoming two decades and also found that how and where this increase will take place. FAO estimated that in the world livestock industry is expected to increase by at least 85% in the year 2030 (FAO, 2011)

It is estimated by United Nations that increase in human population between 2010 and 2050 will double in at least 30 developed and under developing countries of the world. Pakistan is one of them which is expected to have increase in population around 335 million by the year 2050. Globally population of the more developed countries will minimally change. In china demand for meat and meat products has been increased from 9kgs per person to above than 50kgs per person in 30 years only. In other developing countries consumption of meat is a modest 16 kg per person. In Pakistan demand of meat for one person was 14.5 kg in 2007 which was less than the demand in 1995, 14.5 kg per person. In the industrialized countries consumption is around 90 kg per person (Brad and Ismail, 2012).

A research conducted by Anonymous shows that people of developed countries use 80-96 g of proteins per day and in developing countries available proteins for humans are 70 g (Anonymous, 2003). Like other countries in the world, poultry meat in Pakistan also known as important source of food. In year 2014 and 2015 about 28.0% of the total meat was contributed by poultry industry with the annual growth rate of 8-10% which was around 200 billion (The Ministry of Finance, 2015).

Due to increase in the demand of meat, people use heavy metals such as arsenic in the feed of animals to increase the rapid production of animals especially in the poultry industry (Anonymous, 2003). Globally many studies have been conducted to estimate the level of heavy metals in feed and organs of animals and results showed the mixing of large amount of heavy metals in both feed and organs of animals. In poultry industry heavy metals are being used excessively (Abdullah *et al.*, 2001). There are many heavy metals which play important role in human body but arsenic is one of them which has no positive role in human body it only poses health risk for both animals and human. It transferred through the food chain and accumulates in animal tissues, kidney and liver. (Miranda *et al.*, 2003).

The sources of contaminated substances in tissues of meat includes animals drugs, pesticides, fertilizers, feed and other chemicals released by industries or agricultural sites (Fathy *et al.*, 2011). Meat processing in the slaughter houses and farms is also the major reason of arsenic contamination in meat, reported by many researchers (Akan *et al.*, 2010; Harlia and Balia, 2010). During meat processing, removal of hair with the help of flame which fuelled by different substances like wood mixed with engine oil and plastic mixed with tires. These substances consist of contaminated material like arsenic which directly go in to the meat and make it unfit for human diet (Okiei *et al.*, 2009). Another source of contamination in the meat is rearing of animals in the arsenic rich area (Fathy *et al.*, 2011).

One of the major reasons of food contamination, especially in meat, is the improvement in the food production systems and processing technologies. When animals eat this advanced feed, deposition of heavy metals residues take place in meat. Most of the animals graze on fields or land full of arsenic which increases the chances of arsenic accumulation in beef and mutton (Sabir *et al.*, 2003).

Broilers are available from the hatcheries in the form of day-old chicks. These are grown on special feed during their life span. Of the feed ingredients, macro feeding stuffs include cereals, pulses, poultry byproducts, oilseeds, fish and fish by-products, whereas micro-feeding stuffs comprise antibiotics, arsenicals, vitamins, and minerals (Mumtaz *et al.*, 2000). These are being used as feed additives in broiler rations to promote growth and to improve the yellow color of the skin and shanks. Any dietary source of arsenic should be withdrawn from the feed at least 5 days prior to slaughtering. Otherwise the residues may remain in the tissues which may cause problems to human beings (Ensminger, 1980).

For the assessment of residual level in the food, an accurate and vigorous method is required which make sure the sustainability, compliance, maximum production and food safety also. The technique used for the measurement, must ensure the presence of contaminated substance and micronutrients and also recognize that whether the source of contamination is processing, packing or the cooking of the food in the utensils, full of harmful residuals. Genuineness and originality of detection are also compulsory. Inductively coupled plasma mass spectrometry (ICP-MS) is the technique which fulfills all the requirements needed for the assessment. (http://www.Foodquality andsafety .com/article/icp-ms-for-detecting-heavy-metals-in-foodstuffs/).

For the toxic metal determination, two steps are performed usually. First step is of sample digestion and second step is of detection. In the laboratories different methods of sample digestion has been used such as: conventional oven commonly used for the dry ashing, strong acids and microwave digestion is also very common, another acid digestion is performed in the pressure vessel and some scientist directly dissolve the sample into the acid. Some laboratories also extract the metals in 2-methylhexan-2-one [isobutyl methyl ketone (IBMK)]. For the detection of the sample most commonly used technologies are atomic absorption spectroscopy (AAS) and Inductively Coupled Plasma Mass spectroscopy (ICP-MS). Atomic Absorption Spectrometry consists of flame AAS (CVAAS) for the detection of mercury and also contains hydride generation (HG), both used for arsenic detection. In Inductively Coupled Plasma technology, mass spectrometry (ICP-MS) and emission spectrometry (ICP-OES) are available for the determination of contamination. There are some other techniques which also used for the determination for example anodic stripping voltammetry, colorimetry, spectrophotometry, ion chromatography, polarography and titration. But now days these techniques are not used in the laboratories because ICP-MS and AAS have replaced them (Food Safety Authority of Ireland, 2009).

Problem statement

The presence of arsenic in the food, water, soil and air can cause a lot of occupational and environmental health problems. On the bases of many evidences, IARC, a research center on cancer, recognize arsenic and its compounds as carcinogenic to humans (IARC. 1973, 1980). A report presented by Wallinga in 2006 found that arsenic is the major source of many diseases for example, heart attack, diabetes and decline in intellectual functioning (wallinga, 2006).

Ingestion, inhalation and dermal contact are the main routes of arsenic concentration in the body. Currently a lot of food present in the markets which contain arsenic such as fish, shellfish, meat, poultry, dairy products and cereals. Ingestion of food contributes most to the daily intake of arsenic. Drinking water is also the main route of arsenic in humans and animals. Even low dose of arsenic present in the body, absorbed in stomach and intestine then in systematic circulation. After absorption, it metabolized by liver to less toxic form and released by kidney in urine. During this process if the exposure is large then it leads to the accumulation in all parts of the body (Verma *et al.*, 2016).

For the purpose of food safety and human health, arsenic contamination in meat and meat products is of great concern due to highly toxic nature of this metal (Santhi *et al.*, 2008). The livestock which includes sheep and cattle, reared on the pasture lands freely, are the source of environmental pollution (Gallo *et al.*, 1996). Advanced technology found that sometimes arsenic displays its harmful effects in such a way that food regulators don't take it seriously while setting the safe standard level, for example disrupting the hormonal functioning. Severe attacks of arsenic occur on the children hormones which are important for the normal functioning, development of the child brain, gonads and other organs (Wallinga, 2006). Ingestion of arsenic in the body chronically causes the adverse effects for example skin manifestation, vascular disease, renal diseases neurological diseases and cardiovascular diseases, reproductive effects, different kinds of cancer also. Diabetes is also the main health problem accrued by the arsenic exposure (Rahman *et al.*, 1998; Wang *et al.*, 2003).

This study has been designed in view of increasing arsenic toxicity in edible meat. Beef, mutton and chicken are extensively used in diet of humans in Pakistan and it is assumed that there are sources of arsenic in their diet which causes different type of diseases such as cancer.

Significance of the study

The number of toxic effects of arsenic species and its transmission from geological assets to ground water and food is the greatest threat to human beings (Ammann, 2011). In the earlier time arsenic was a useful metal which normally used as a rodenticide but continuous low level exposure of this toxic metal caused skin, vascular and nervous system disorders (Food Safety Authority of Ireland, 2009). Due to severe harmful effects of arsenic and lack of researches on it, this is utmost requirement of this time, to conduct researches for the purpose of determination of arsenic in meat, by taking samples from different areas of Pakistan to know the concentration or level of arsenic in meat that humans are consuming daily.

Objectives

1. To determine the As concentration in beef mutton and chicken from four different cities and comparison with permissible limits
2. Evaluate the bio-concentration pattern of arsenic in different animal's protein to determine the health risk.

Chapter # 2

LITERATURE REVIEW

LITERATURE REVIEW

Arsenic is a toxic metal spread widely on the earth as arsenic sulfide or arsenate and arsenide. In the environment this metal releases in the trioxide form with the help of high temperature. It absorbed in the environment specifically on particles such as dust particles which then dispersed in the whole environment through wind (WHO, 2010). It has been used commercially for many centuries in pharmaceuticals, wood preservatives, agricultural chemicals, mining, glass making, metallurgical and in semi conductor industries (WHO, 2001).

Many biological and abiotic factors may enhance the toxic effects of arsenic on environment such as temperature, PH level, Eh, organic content, adsorption to solid matrices, presence of phosphates, other toxicants and the time period of exposure (Eisler, 1988).

Now a day science advances in the analytical techniques a lot, which opens a new way to understand the role of toxic substances in the biological processes that occur in the human body. Living things exposed to toxic metals mainly through the food (Balzan *et al.,* 2004). Intake of food contaminated with toxic chemicals may cause the episodes of intoxication which is known as acute and when a disease displays in latent time period, extended intoxication. Chemicals reach to the threshold limits when entered in the tissues of the body and cause severe illness which may cause death (Cuadrado *et al.*, 2000).

All types of meat present in the world become the important source of food because they contain large amount of nutrients as well as a lot of toxic substances. In the world among all the meat types, chicken meat has the highest rate of consumption per person (Liu *et al.*, 2016). It is estimated by Food and Agriculture Organization that total meat production was 314.7 million out of which 110.2 million tones was of chicken meat in the year 2014 (Imran et al., 2015). In China, annual growth of meat consumption increase at over 5 percent per annum internationally. As population increases red meat demand is also increase globally such as in China and Brazil, not for the reason of larger expendable income (Bradfield and Ismail 2012). In the North America, chicken is the most commonly used meat on a per capita basis and the total meat ingest by a person 17.7 billion kg per year (AAFC, 2013; USDA, 2014).

In Pakistan from the year 1995 to 2011, population growth rate increases rapidly, about 37 million population was in 1950 and in 2011 population was 173 million. As population increases at the same time livestock rate was also rise up from 30 million to above 160 million. In the year 2010, Pakistan's livestock which includes cattle, buffalo and camel, was estimated to 65 million and small livestock was 87 million approximately out of 150 million total animals (Bradfield and Ismail, 2012).

Arsenic entered in the body through three different ways, ingestion, inhalation and dermal contact. Ingestion of three low doses of water and food are the main path ways of arsenic entrance in the living organism after entering they absorbed into different organs and then discharge into the blood stream. Arsenic is then converted into less toxic form and then excreted into urine (Caroli *et al.*, 1996).

Natural as well as anthropogenic activities are the source of contamination in livestock production systems which directly contaminate the meat and meat products. In the agricultural sector fertilizers and agricultural lime is used for the production of plants which contains impurities and some fertilizers used for plants growth, consist of phosphates which accumulates in plants body and soil also then entered in the animals and humans body (Nicholson *et al.*, 2003; Senesi, *et al.*, 1999).

Arsenic affects both acute and chronically when ingested with the contaminated water, food, smoke, wine, drugs, and fossil fuels (NTP, 1999). Arsenic exposed to humans on all the levels such as in occupational places, medical centers and domestically also. In occupational places workers of the industries have been affected by arsenic release through smelting and refining of metal ores, production and use of chemical in industries, manufacturing of glass, pharmaceutical and semi conductor objects (USPHS, 1989). In the field of medicines arsenic affect the humans through arsenic containing drugs which used to treat many diseases like asthama and syphilis (Wong *et al.*, 1998; Ko, 1999). ATSDR in 1998 found that arsenic toxicity in the seafood is in inorganic form which considered as less toxic form. MCL is the concentration standard generates by US EPA, according to it drinking water standard is 10µg/l, above it water is not suitable for drinking purposes (EPA, 2001).

In the body most vulnerable organs are liver, kidney and small intestine, which accumulate higher level of trace metals (Horky *et al.*, 1998).

Currently, a lot of food present in the market in which arsenic used intentionally. Most commonly available feed for chicken also consist of arsenic, when farmers used it for chicken feed, it add more arsenic burden in to the environment. Some people in the poultry industry claims that arsenic used in the feed of chicken for the healthy growth of them. Europe has banned arsenic in animal feeds. (wallinga, 2006).

Khalafalla and his fellows conducted a research to detect the residual level of arsenic, lead and cadmium in the two different organs such as liver and kidney of buffalo. According to the results over all mean residuals of lead were 8.77 Fig/kg, 47.70 Fig/kg and 109.42 Fig/kg fresh weight in muscles and the mean concentration of cadmium was 1.40 Fig/kg and 62.56 Fig/kg fresh weight and arsenic level in meat samples was 5.06 Fig/kg, 4.64 Fig/kg and 14.92 Fig/kg of fresh weight which shows that arsenic concentration in meat samples was above permissible limits and harmful for human use (Khalafalla *et al.*, 2011).

Red meat and other all kinds of meat become contaminated because of multiple sources such as drugs used for animals, feed and waste of fertilizers and chemicals (Pearson e*t al.*, 1990).

Nkansah and Ansah observed that in Ghana, meat of chicken, goat and sheep are the major sources of proteins, vitamins and fats to the population. In this country meat consumption by per person was 9.2 kgs and the level of arsenic in meat was found due to feed of animals and water use for drinking purposes. The actual reason of this research was to detect the heavy metals like Cd, Hg, Cr, Pb and As in meat by using the Varian Atomic Absorption Spectrophotometer. The concentration of arsenic and other metals was different in cow, pig, sheep, deer, goat and grass cutter. The concentration of lead was above the permissible limits in beef and less in pork and other metals were below the permissible limits (Nkansah and Ansah, 2014).

A study conducted in Zambia shows that agricultural practices in Zambia are the major source of arsenic contamination in the area. John investigated the offal of cattle present in this area; consist of agricultural lime, fertilizer and pesticides. Soil and pasture land on which animals graze consist of fertilizers which directly go into the animal body. According to the results highest mean value was determined in the Cu

(40.9), Zn (35.2), Cr (1.35) in the liver samples. In the liver, highest mean values were determined in Cu, Zn and Ni but highest mean values in kidney were of Pb and Cd. Maximum levels of metals like As, Hg, and Co were below 0.2 mg/kg in liver and kidney (Yabe *et al.,* 2012).

Another study conducted by Akan *et al.,* found the concentration of toxic metals in the liver muscles and kidney of beef, mutton, caprine and chicken. Among all the heavy metals, level of arsenic was below the permissible limits but Cr and Pb were above the permissible limits. The results were statistically significant. The main reason of little amount of arsenic present in meat was the exposure of animals to the arsenic rich water, but still the level was not above the threshold limit which indicates that people can easily use it in their diet without any harm (Akan *et al.,* 2010).

Mariam *et al.,* 2004 concluded that the high levels of heavy metals in the liver, kidney and lean meat of beef, mutton and poultry samples collected from different markets of Lahore vouched for the increasing levels of environmental pollution.

A report published in 2006 by the IATP, USA, for the determination of arsenic in chicken meat, sold in California and Minnesota. For the detection of arsenic inductively coupled plasma mass spectroscopy was used and samples were prepared for detection, by digestion process. The total number of samples was 155 collected from supermarket. The results found that most of the meat used by people consists of arsenic. According to a report roxarsone, a feed additive given to chicken is about 1.7 to 2.2 million pounds in a year. Arsenic subsequently contaminates much of the 26-55 billion pounds of litter or waste generated each year by the U.S. broiler chicken industry, likely also contaminating the communities where that waste is generated or dispersed.

From total 155 samples, no arsenic residuals were present in 45% samples or they were under the permissible limits (wallinga, 2006).

A study conducted by Lasky *et al*, to assess the toxic level of arsenic in liver and muscle of chicken and also to found the inorganic arsenic quantity present in the human body just because of consuming chicken. For the estimation of arsenic toxicity in human, lasky and his fellows used the monitoring data obtained from Food Safety and Inspection Services, for the year 1994-2000. The concentration of arsenic in chickens was 0.39ppm, which is about 3-4 folds larger than in poultry, mutton and beef meat. At the level of meat people intake a lot of inorganic arsenic from chicken meat alone (ingested meat is 60 g per person and arsenic content intake daily through meat is 1.38-5.24 µg/day). The reason of higher concentration of arsenic in chicken meat was the use of arsenic feed additives in the meal of chicken which directly goes into the human body. (Lasky *et al.*, 2004).

In the year 2013 Nachman and his fellows conducted a research to differentiate the types of arsenic in chicken and also estimate the risk connected with the bladder and lungs cancer due to consumption of chicken, produced with arsenic containing feed. For the research purpose, two types of chicken samples were collected from the market, one is antibiotic free and other consists of organic arsenic. The samples were collected from the super stores of ten different areas of US. In the time period of June, December 2010. For the testing 116 samples in the raw form and 78 cooked meat samples were collected. The result found that total geometric arsenic mean in cooked meat was 3.0 g/kg (95% CI: 2.5, 3.6). In the 78 samples arsenic concentration was high

(GM = 1.8 g/kg). In 20 – 40 conventional samples roxerson was determined (Nachman *et al.*, 2013).

A study carried out by Johan and Jean in 1994 was estimated the toxicity of arsenic, cadmium, mercury and lead in muscles goats. The results found that the toxicity level of all the selected metals was highly above the permissible limits and not suitable for eating purposes (Johan and Jean, 1994).

A study conducted by Imran and his fellows shows that level of arsenic in poultry meat samples collected from Kasur, Pakistan, was above the permissible limits and is not suitable for eating purposes. Kasur city is famous for the tanning industry that's why this area was selected for the sample collection. Different organs of chicken was collected, 11 samples of heart, 6 samples of kidney and 12 samples of leg tissues, wings, leg bones, wing bones, backbone, ribcage and neck. Samples were analyzed for the arsenic, cadmium, copper, chromium, mercury, iron, lead, manganese, nickel and zinc. Inductively Coupled Plasma- Spectrophotometer was used to analyze the sample. The level of arsenic was under the permissible limits when compare with the reference material. Cadmium and lead was exceeding the permissible limits set by ANZFA. Copper, chromium and zinc were also under the permissible limits. Manganese was above the threshold limit set by WHO (Imran *et al., 2014*).

A study conducted by Ghosh *et al.,* shows the correlations of arsenic residuals in different organs of body, thigh muscle, liver, kidney, skin, lungs and excreta of broiler chicken. It was estimated that all the arsenic content was entering in the chicken body through drinking water and feed as well. Result displays the level of arsenic in excreta and tissues of chicken correlated to the level of arsenic in drinking water. In the same way significant positive correlations were found in level of arsenic in chicken and feed (Ghosh *et al.*, 2012).

In the current time period the demand of poultry meat is increasing frequently which influenced its production significantly. To increase the production of the meat, people used modified feed for more production of chicken. It is reported by Ismail that whatever the source of modified feed production, every source consist of heavy metals content in them which directly harm the animal as well as humans (Bradfield and Ismail, 2012).

Xie in 2004 fed chicken, both organic and in organic arsenic. According to the results liver is the organ which rapidly accumulates the both forms of arsenic, liver is the organ which generates detoxification. So it proves that both forms of arsenic can easily accumulates in liver and become toxic for the animals and human also (Xie *et al.*, 2004).

A research conducted by Mohammad and his fellows in 2013 conclude the concentration of heavy metals such as As, Cr, Cd, Fe and Pb in the tissues of different organs such as muscle, gizzard, liver and kidney of two different chicken species. The samples were collected from two different poultry farms in the year 2012. For the assessment of samples Inductively Coupled Optical Emission Spectroscopy was used. The findings showed that residuals of arsenic were present in the order: breast meat > kidney > gizzard > liver. This study proves that adult layer chicken were safe for human consumption (Mohammad *et al.*, 2013).

Many other studies have been conducted by researchers to find out arsenic concentration in chicken meat samples such as by Batista *et al.*, 2012; Doyle Spaulding 1978; Jeinek and Corneliussen 1977).

Toxicology fact sheet series issued by food safety authority of Ireland in 2009 shows that arsenic occurrence in sea animals comprises of above 90% of total food. According to the findings brown meat has less arsenic content as compare to white meat and in sea crab is the sea animal in which high level of arsenic found. Other animals such as shellfish, molluscus and seaweeds have less residuals of arsenic than crabs. In fish the type of arsenic which mostly present is arsenobetaine, a type in which arsenic is considered to be nontoxic (Food Safety Authority of Ireland, 2009).

The JECFA has suggested the provisional daily intake of inorganic arsenic for a 60 kg young man which was about 0.002 mg/kg of body weight. Fish and the fish products considered to be full of arsenic but in less toxic form, normally in organic form (Food Safety Authority of Ireland, 2009).

It is evident that in aquatic animals cytotoxicity increase in fish cell lines when they exposed to sodium arsenite, found by Wang and his fellows and Seok (Wang *et al.*, 2004; Seok *et al.*, 2007. It was also observed by Bhattacharya and Ventura and his fellows that oxidative stress and decrease in anitioxidant enzymes in fish and other sea animals occur due to exposure of them to the As_2O_3 (Bhattacharya, 2007 ; Ventura-Lima *et al.*, 2007). Evidence proves that if zebrafish exposed to arsenate for two days only, the antioxidant responses in gills increases (Ventura-Lima *et al.*, 2009). A study conducted by Bagnyulova *et al*, 2007 shows oxidative stress and modulation of the antioxidant system in liver of goldfish exposed to arsenite (Bagnyukova *et al.*, 2007).

Globally, due to advances in technologies and also due to the increased arsenic exposure allowed scientist to research on the issues of arsenic. All the researchers conclude that drinking water, environment and feed is the main source of arsenic intake inn animals and human body (John and Jean, 1994; Miranda *et al.*, 2005; Sharif *et al.*, 2005; Jukna *et al.*, 2006; Rudy, 2009; Smith *et al.*, 2010; Poti *et al.*, 2010; Szkoda *et al.*, 2002; Hanan *et al.*, 2012; Tabinda *et al.*, 2013).

Johann and Jean conducted a research to determine the residuals of arsenic, cadmium, chromium, cobalt, copper, lead, manganese, mercury, molybdenum, nickel, selenium and zinc. Atomic absorption spectrophotometry was used to determine the residuals in the livers and kidney of 190 cattle selected from 8 different points located in South Wales. Among all the trace metals mean and range value of arsenic was 0.013 (n.d.-0.09). the level of arsenic was significantly different in the trace metals levels in liver as well as in kidney from different regions of the State and the trace metals level in liver and kidney in the same region. NBS No 1577 Bovine Liver was used to check the accuracy of the methods (Johan and Jean, 1994).

Nicholson and Senesi found that heavy metals such as Cd, Pb, Hg and As are the metals which rapidly enters into the food chain and generates serious health risk for humans and animals also. Metals exposed to the human through many sources such as natural and anthropogenic. In agricultural systems significant sources of metal productions are contamination due to livestock production and intensive agricultural practices (Nicholson *et al.*, 2003; Senesi *et al.*, 1999).

Arsenic is not harmful for every person on the same rate; degree of effect for children, adults and older persons is different. For the adults effect of arsenic depends on the perfection of immune system of the body, metabolization and their generic make up also. In children arsenic effect quickly because of less potential to fight with the germs, very low amount of arsenic in children can create serious problem. So children are more vulnerable (NRC, 2001; Mead, 2005). There is very less research on the effect of arsenic in children. Some studies have suggested that arsenic effect on the birth also, create birth defects. Some studies on human displays the effect of arsenic containing drinking water on the reproduction system and cause miscarriage, still birth and preterm birth (NRC, 2001).

Exposure of inorganic form of arsenic in the raw form or in refined form both causes rhinopharyngo-laryngitis and pulmonary insufficiency (WHO, 1981). Instead of inhalation, arsenic entered in the body through any other route, can directly damage the respiratory tract and also cause chronic cough (Borgono *et al.*, 1977).

Many epidemiological studies reveal that skin disorders are occurring now days due to arsenic exposure to the humans. Fresh water that contains arsenic concentration of 0.01-0.1 mg/kg/day can cause hyperkerotosis, corns on the palm and foot and hyperpigmentation on the portion of neck, back and face (Saha *et al.*, 1988).

Chapter # 3

MATERIAL AND METHODS

MATERIAL AND METHODS

Study Area

The present study was carried out in four different cities of Pakistan viz., Peshawar, Islamabad, Gujrat and Lahore for arsenic assessment in different types of meat samples. The people of these cities consume extensive amount of different types of meat in their daily diet. Islamabad is the capital of Pakistan lies between 33.72°N and 73.09°E with the population of 805235 (Census report of Pakistan, 1998). Lahore is the capital of Punjab province lies between 31.58°N and 74.32°E with the population of 6318745 (Census report of Pakistan, 1998). Gujrat is the 18th largest city of Pakistan situated in Punjab province and lies between 32.57°N and 74.07°E with the population 2048008 (Census report of Pakistan, 1998). Peshawar is the capital of Khyber Pakhtunkhwa lies between 34.01°N, 71.58°E with the population 988055 (Census report of Pakistan, 1998).

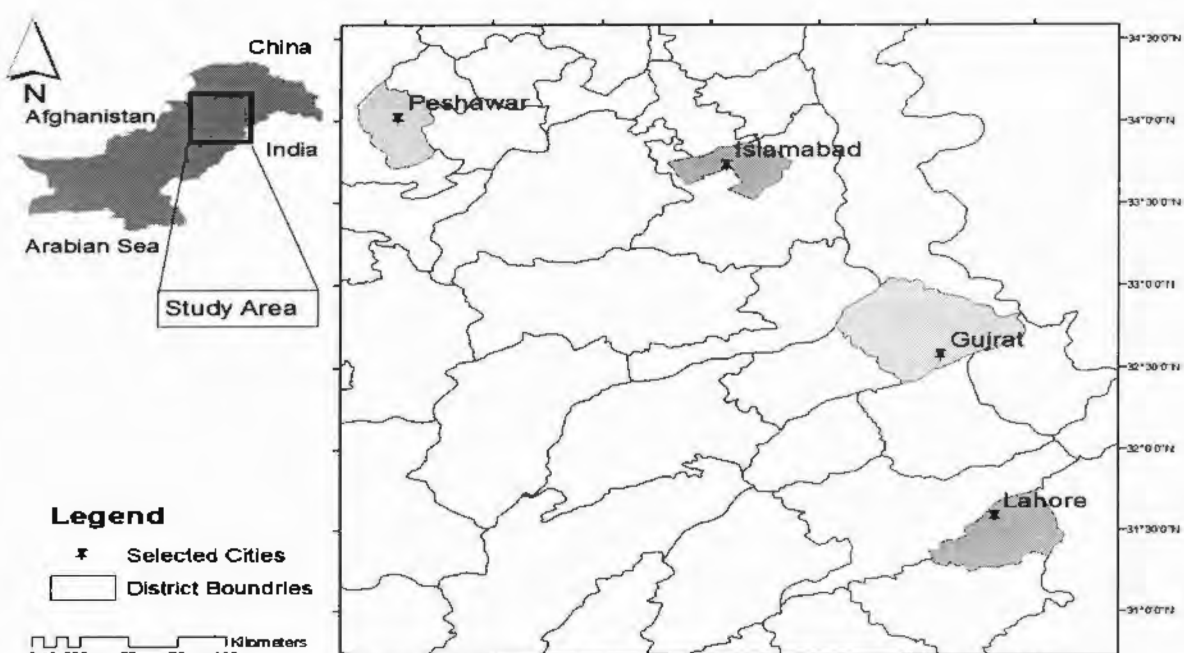

Figure 1: location map of study area

Collection of Material

Seventy two fresh samples of muscle and liver of chicken, mutton and beef were collected from different shops of the respective cities (Fig 1). From each city nine liver samples of chicken, mutton and beef were collected and also nine muscle samples of chicken, mutton and beef were collected. The weight of each sample was ranged between 150 to 250 grams. After collection, samples were immediately preserved in an ice box and transferred to the laboratory where they were classified, weighed and kept frozen at -20°C until further analysis.

Decomposition of Meat Samples for Mineral Analysis (Arsenic)

For arsenic analysis, samples were washed with distilled water; irrelevant tissues and fats were removed from samples and then chopped into small pieces with the help of knife. For moisture removing, all samples were weighed and placed in the electric furnace at 60°C, for 24 hours approximately. After drying, all the samples were reweighed to check the moisture difference in order to avoid moisture in the collected samples. Powdered form of samples was obtained by grinding them in the grinding machine then (2gs of each sample) were sealed in the polyethylene bags labeled with identification code and preserved at -20°C before digestion.

Meat samples weighed individually (about 1 g), two mL GR v/v HNO_3 and $HCLO_4$ was added in them and put into digestion tubes (CNW Corporation, Shangai, China) overnight and on the second day mix 1 mL H_2O_2 GR grade 30% (v/v) (Sinopharm Chemical Reagent Co., Ltd, Beijing, China). After mixing, digestion tubes were sealed and digested in water bath at 100 C° for 12 hours. The water volume of the digested samples was raised to 50 mL by using Milli Q and finally filtered through 0.22 μm nylon membranes (Alamdar *et al.*, 2016).

The prepared digests (samples) were analyzed in Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Bazilio *et al.*, 2012). The commercially available reference material from international Atomic Energy Agency (IAEA) was used to check the accuracy of the analysis. For meat IAEA 434 (phosphogypsum 1.98±0.21 mg/kg) was used and recovery rate was 90-102%. Metal concentration in meat samples was expressed in parts per billion (ppb).

Statistical analysis

Basic descriptive statistics of arsenic in meat samples was performed with the help of Microsoft Excel. Two way ANOVA was also used to estimate the statistically significant difference in mean arsenic concentration in liver and muscles of animals. In Two way ANOVA dependent variables were localities, organs and animals and independent variable was arsenic concentration. Significant level was considered as $P \leq 0.05$ or less.

Average Daily Intake and The Human Health Risk Assessment

The average daily intake of arsenic by the humans was calculated by using the following formula suggested by USEPA (USEPA, 1997).

$$EDI = \frac{C \times IR \times EF \times ED}{BW \times AT}$$ (Zhaung *et al.*, 2014)

In this equation EDI stands for the average daily intake (μg/kg/day); C consist of arsenic concentration in the exposure medium (ppb or ppm); IR is the ingestion rate (0.07 μg/kg bw/day); EF is the exposure frequency which was 365 days/year; ED represents the exposure duration (70 years); BW is the average body weight in Pakistan (kg) and AT is the duration over which the dose is averaged (assumed to be 70) (Zhaung *et al.*, 2014).

The Human Health Risk was calculated with help of the following equation:

$$THQ = \frac{EDI}{RfD}$$ (Zhaung *et al.*, 2014)

In this equation THQ stands for The Human Health Risk, EDI presented the Average Daily Intake and RfD shows the Reference Dose which was 0.3 ppb suggested by USEPA (USEPA 1991a).

Chapter # 4

RESULTS

RESULTS

Spatial Distribution of Arsenic Concentration

The arsenic present in liver and muscle samples of different animals via chicken, Goat and Cow collected from representative cities (Lahore, Gujrat, Islamabad and Peshawar) of Pakistan has been summarized in Table 1 and 2. The residual level of arsenic in Lahore was higher in the liver samples of goat (126.90 ± 55.79 ppb), ranged between 64.45ppb to 171.85ppb. In the chicken (27.48 ± 14.52 ppb) and cow (26.72 ± 7.71 ppb) arsenic content was also present but in low quantity (table 1). In cow lowest concentration of arsenic was present which ranged from 18.02ppb to 32.69ppb. The concentration of arsenic in Gujrat was higher in liver samples of cow (91.62 ± 73.86ppb), ranged from 39.40ppb to 143.85ppb. The lowest concentration was present in chicken samples (32.47 ± 3.30ppb), ranged between 28.66ppb to 34.61ppb (table 1). Goat liver samples in Gujrat also consist of arsenic. Arsenic concentration in the liver samples collected from Islamabad showed highest concentration in the goat (100.44 ± 5.74ppb), ranged from 96.81ppb to 107.06ppb. The lowest concentration of arsenic in liver samples of Islamabad was in chicken (41.50 ± 29.99ppb), ranged between 20.05ppb to 75.78ppb (table 1). Arsenic level in goat liver samples was also high. The residuals of arsenic in the liver samples collected from Peshawar showed highest level in the cow (137.41 ± 24.37ppb), ranged from 117.26ppb to 164.51ppb. The lowest concentration was present in goat (27.62 ± 6.24ppb), ranged from 22.16ppb to 34.43ppb (table 1).

The residual of arsenic in the muscle samples collected from Lahore showed highest content in goat (106.86 ± 44.46ppb), ranged from 66.57ppb to 154.56ppb. Other samples of Lahore were also contaminated with the arsenic concentration but the lowest concentration was present in the chicken (60.60 ± 32.61ppb), ranged from 28.11ppb to 93.34ppb (table 2). Muscle samples collected from Gujrat displays higher level of arsenic in chicken (228.38 ± 46.10ppb), ranged from 195.77ppb to 260.98ppb. Residual of arsenic was present in other samples also but the lowest was in goat (76.08 ± 32.92ppb), ranged from 40.68ppb to 105.80ppb (table 2). Samples of muscles collected from Islamabad showed highest concentration of arsenic in cow (52.33 ± 9.72ppb), ranged from 42.14ppb to 61.50ppb. The lowest concentration was present in goat (34.37 ± 11.54ppb), ranged from 21.58ppb to 44.01ppb (table 2). The concentration of arsenic in the muscle samples of Peshawar displays the highest concentration in the cow (205.73 ± 149.96ppb), ranged between 32.61ppb to 295.86ppb. The lowest concentration in muscle samples of Peshawar was in chicken (45.09 ± 14.52ppb), ranged between 33.58ppb to 61.42ppb (table 2).

Source Distribution of Arsenic Concentration

The residual level of arsenic was significantly higher in chicken muscle samples of Lahore with the mean value of 60.60 ± 32.61 ppb ranged from 28.11 - 93.34 ppb than in liver with the mean concentration of 27.49 ± 14.52 ppb ranged from 18.95 - 44.25 ppb. In chicken samples of Gujrat, concentration of arsenic was above in muscle samples with the mean value of 228.38 ± 46.10 ppb, ranged from 195.77- 260.98 ppb as compare to liver 32.47 ± 3.30 ppb ranged from 28.66 - 34.61 ppb. In Islamabad muscle samples of chicken showed higher value of arsenic 49.61 ± 12.18 ppb ranged from 37.41 – 61.79 ppb as compare to liver 41.50 ± 29.99 ppb ranged from 20.05 – 75.78 ppb.

Table: 1 Two way ANOVA showing mean ± SD, minimum and maximum arsenic concentration (ppb) in liver samples of different meat types collected from different localities.

Locality	Animal	Mean ± S.D	Minimum-Maximum	Permissible limits	
				ANZFA	China's Maximum Levels for Contaminants in Foods.
Lahore	Chicken	27.48 ± 14.52	18.95 - 44.25	2.0ppm (2000ppb)	0.5ppm (500ppb)
	Goat	126.90 ± 55.79	64.45 - 171.85	-	
	Cow	26.72 ± 7.71	18.02 - 32.69	-	
Gujrat	Chicken	32.47 ± 3.30	28.66 - 34.61	2.0ppm (2000ppb)	0.5ppm (500ppb)
	Goat	85.52 ± 72.27	22.41 – 164.37	-	
	Cow	91.62 ± 73.86	39.40 – 143.85	-	
Islamabad	Chicken	41.50 ± 29.99	20.05 – 75.78	2.0ppm (2000ppb)	0.5ppm (500ppb)
	Goat	100.44 ± 5.74	96.81 – 107.06	-	
Peshawar	Cow	51.17 ± 28.64	29.55 – 83.65	-	0.5ppm (500ppb)
Peshawar	Chicken	54.14 ± 50.65	20.61 – 112.41	2.0ppm (2000ppb)	0.5ppm (500ppb)
	Goat	27.62 ± 6.24	22.16 – 34.43	-	
	Cow	137.41 ± 24.37	117.26 – 164.51	-	

Table 2 Two way ANOVA showing mean ± SD, minimum and maximum arsenic concentration (ppb) in muscle samples of different meat types collected from different localities.

Locality	Animal	Mean ± SD	Minimum-Maximum	Permissible limits	
				ANZFA	China's Maximum Levels for Contaminants in Foods.
Lahore	**Chicken**	60.60 ± 32.61	28.11 - 93.34	2.0ppm (2000ppb)	0.5ppm (500ppb)
	Goat	106.86 ± 44.46	66.57 - 154.56	-	
	Cow	76.28 ± 30.85	43.41 - 104.61	-	
Gujrat	**Chicken**	228.38 ± 46.10	195.77- 260.98	2.0ppm (2000ppb)	0.5ppm (500ppb)
	Goat	76.08 ± 32.92	40.68 – 105.80	-	
	Cow	134.43 ± 124.96	59.40 – 278.69	-	
Islamabad	**Chicken**	49.61 ± 12.18	37.41 – 61.79	2.0ppm (2000ppb)	0.5ppm (500ppb)
	Goat	34.37 ± 11.54	21.58 – 44.01	-	
	Cow	52.33 ± 9.72	42.14 – 61.50	-	
Peshawar	**Chicken**	45.09 ± 14.52	33.58 – 61.42	2.0ppm (2000ppb)	0.5ppm (500ppb)
	Goat	165.49 ± 124.62	28.10 – 271.24	-	
	Cow	205.73 ± 149.96	32.61 – 295.86	-	

In Peshawar, liver samples of chicken showed more arsenic 54.14 ± 50.65 ppb ranged from 20.61 – 112.41 ppb as compare to muscle 45.09±14.52 ppb ranged from 33.58 – 61.42 ppb.

Mutton samples collected from Lahore showed significantly high level of arsenic in liver with the mean value of 126.90 ± 55.79 ppb ranged from 64.45 - 171.85 ppb as compare to muscle 106.8±44.50 ppb ranged from 66.57 - 154.56 ppb. Samples collected from Gujrat, Islamabad and Peshawar showed high concentration of arsenic in muscle samples (152.2±135.8 ppb, 49.61±12.18 ppb and 165.4±124.6 ppb) as compare to liver (115.8±67.02 ppb, 41.50±30.00 ppb and 27.63±6.24 ppb).

Beef samples collected from different cities showed high level of arsenic in muscle as compare to liver. In Lahore muscle samples ranged from 43.41 - 104.61 ppb with the mean value of 76.28 ± 30.85 ppb. In Gujrat muscle samples ranged from 59.40 – 278.69 ppb with the mean value of 134.43 ± 124.96 ppb. In Islamabad also muscle samples of beef were more influenced by arsenic with the mean value of 52.33 ± 9.72 ppb ranged from 42.14 – 61.50 ppb and in Peshawar muscle samples of beef showed high level of arsenic with the mean value of 205.7±149.9 ppb ranged from 32.61 – 295.86 ppb.

The data assessment through two way ANOVA showed the statistical significance level at probability of 0.05 between organs (liver and muscle), animals (chicken, goat and cow) and localities via Lahore, Gujrat, Islamabad and Peshawar (table 3). The F value of localities was 3.55 and P value was 0.02 displayed by ANOVA, less than the significant level which shows that the all the samples from different localities were contaminated with arsenic residuals and the residual level varies. The F value of among organs was 5.61 and P value was 0.02 which was below the significant level. It depicts that different organs such as liver and muscles collected from different areas showed concentration of arsenic and the data showed variation also. The F value between animals source was 9.39 and P value was 0.00 which shows that the level of arsenic is statistically significant. The residual of arsenic was present in all the animal sources in high quantity (table 3).

Table 3 Two way ANOVA showing variation in arsenic concentration between localities, organs and animals.

Source	F value	P value
Localities	3.55	0.02
Organ	5.61	0.02
Animals	9.39	0.00

Probability is $p \leq 0.05$

Arsenic concentration in the liver samples of chicken collected from different localities showed that in chicken samples few concentrations exist below the median value and small data deviate from the median. In the chicken samples one outlier formed which showed that concentration is very high and one value is very extreme. In the liver samples of mutton one outlier formed and one value is very extreme. The data in mutton values were above the median only few values were below. In the beef samples no outlier and extreme values were found but a lot of data deviate from first and second quartile. The order presented: Beef > Mutton > Chicken

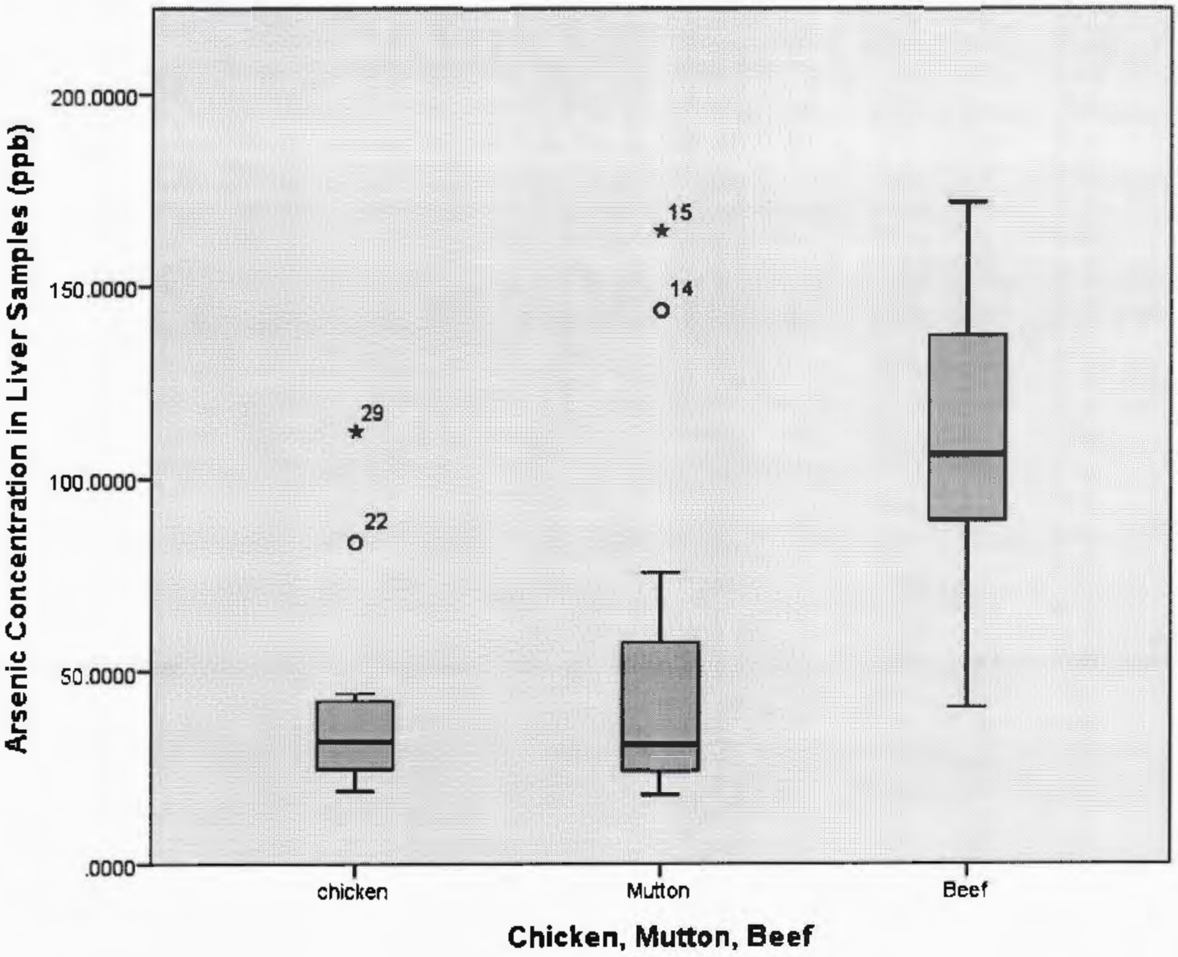

Figure 2: Box and Whiskers Plots of Arsenic Concentration in Liver Samples Regarding Source (Chicken, Mutton and Beef)

In the muscle samples of chicken, mutton and beef collected from different localities in Pakistan showed variation in the residual level. In the chicken samples maximum values were above first quartile and few values were above median. A lot of data deviate from first and second quartile but no outliers and extreme values were formed. In the mutton samples maximum values were above the median and a lot of values deviate from the quartiles. In the beef samples few values exist below median but data deviate from the quartiles. The maximum residuals of arsenic were found in the muscle samples of mutton and beef. The order was found: Beef > Mutton > Chicken.

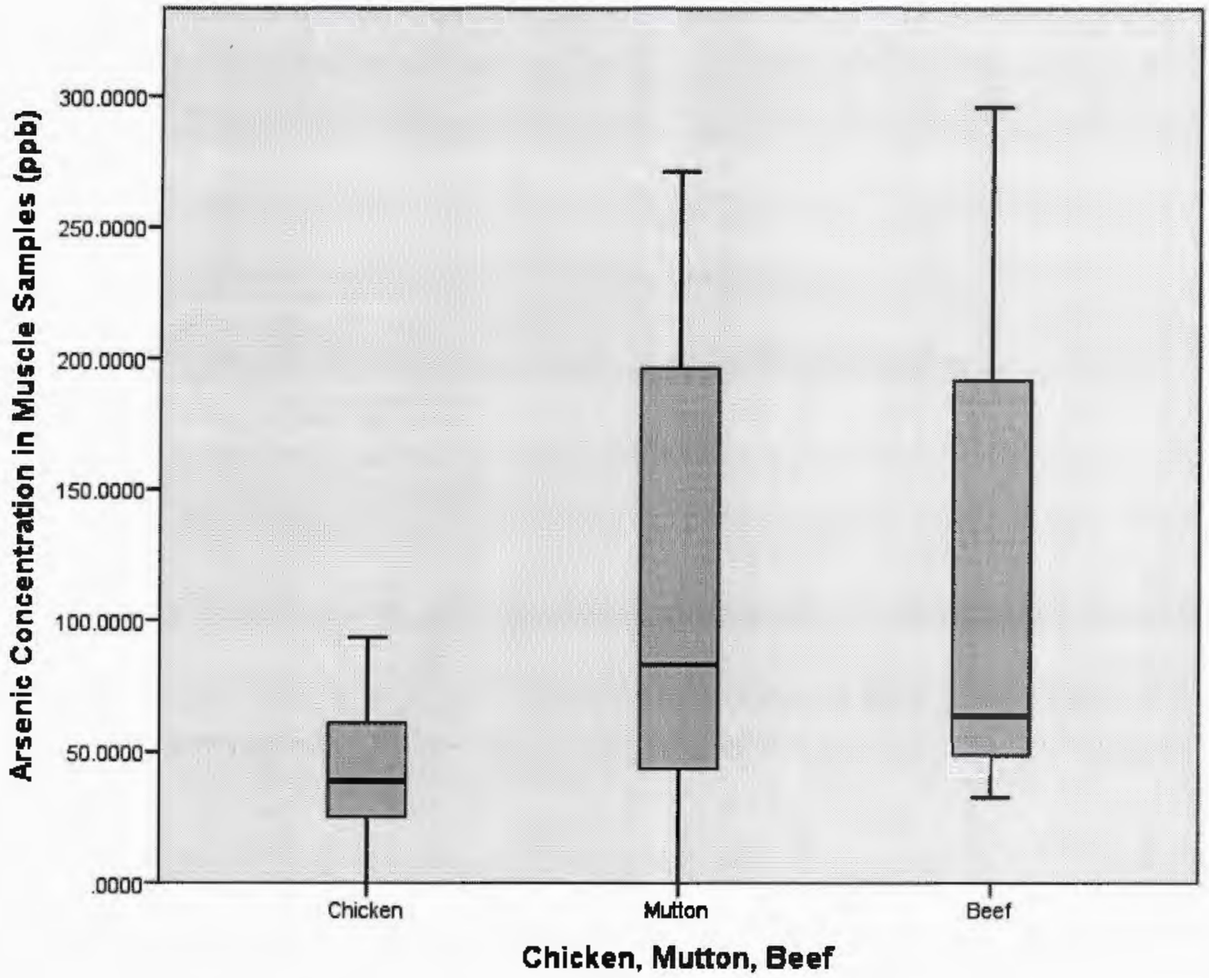

Figure 3: Box and Whisker Plot showing Arsenic Concentration in Muscle Samples Regarding Source (Chicken, Mutton, Beef)

When the value of THQ is less than 1, the toxic effects assumed to be very low but when the value exceeds 1, than there are a lot of toxic effects of the representative metal such as arsenic (Zhuang *et al.*, 2014). In the current study THQ of some meat samples was below the threshold limit (1) and some values were below the limit. Table 4 is showing the EDI and THQ for human consumption of meat. Even low quantity of arsenic daily ingested by an individual can create great health risks for human beings.

Although concentration in all the samples was under the permissible limits but still poses serious health effects.

Table 4 Average Daily Intake and The Human Health Risk of Arsenic.

Localities	Animal	Organ	Mean value (ppb)	Average daily intake by Humans (µg/kgbw/day)	The Human Health Risk (THQ)
Lahore	Chicken	Liver	27.48	0.002	0.06
		Muscle	60.60	0.04	1.6
	Goat	Liver	126.90	0.09	3.26
		Muscle	106.86	0.08	2.72
	Cow	Liver	26.72	0.01	0.33
		Muscle	76.28	0.05	1.66
Gujrat	Chicken	Liver	32.47	0.02	0.81
		Muscle	228.38	0.17	5.98
	Goat	Liver	85.52	0.06	2.17
		Muscle	76.08	0.05	1.90
	Cow	Liver	91.62	0.07	2.33
		Muscle	134.43	0.10	3.53
Islamabad	Chicken	Liver	41.50	0.03	1.0
		Muscle	49.61	0.03	1.0
	Goat	Liver	100.44	0.08	2.72
		Muscle	34.37	0.02	0.81
	Cow	Liver	51.17	0.04	1.33
		Muscle	52.33	0.04	1.33
Peshawar	Chicken	Liver	54.14	0.04	1.33
		Muscle	45.09	0.03	1.0
	Goat	Liver	27.62	0.01	0.5
		Muscle	165.49	0.13	4.33
	Cow	Liver	137.41	0.10	3.33
		Muscle	205.73	0.16	5.33

Chapter# 5

DISCUSSION

DISCUSSION

The presence of arsenic even at low level in meat samples showed exposure of animals to the arsenic containing environment and food. Meat samples showed variation in arsenic concentration in different cities which indicates the difference in uptake, storage and accumulation pattern in animals. The results found that the liver samples of chicken collected from Peshawar showed high concentration of arsenic (54.14±50.65 ppb) as similar results were obtained by Mariam *et al.*, 2004 and Wallinga, 2006. In the muscles samples of chicken from all the selected cities (fig 1), highest concentration was recorded in Gujrat (228.38 ± 46.10 ppb) as found by Ghosh *et al.*, 2012 and Liu et al., 2016. High residual of arsenic in liver and muscle samples of mutton were recorded in Lahore and Peshawar (126.90 ± 55.79 ppb and 165.4±124.6 ppb), similar results were found by Mariam *et al.*, 2004. Liver and muscle samples of beef showed high level of arsenic in Peshawar (137.41±24.3 ppb and 205.7±149.9 ppb) also determined by Mariam *et al.*, 2004 and Khalafalla *et al.*, 2011. Arsenic in beef and mutton tissues may occurred due to variety of sources such as drugs of animals, pesticides, fertilizers, feed consist of arsenic and industrial chemicals that discharge directly into the water (Mariam *et al.*, 2004). A study conducted by chakroborti in kolsur village, India found that cattle effected by arsenic because of water and food sources as these animals drink average 40 liters of water and eat 4 to 5 kgs of hay per day and about 8 fields comprises of 1900µg of arsenic in every kg of hay (Chakroborti, 2001). Different studies have been conducted by researchers which indicate the presence of arsenic in groundwater, above the permissible limits, used as water source for animals. The maximum reported level of ground water containing arsenic from kalanwala, Punjab province is about 1900 µg/L (Farooqi e*t al.,* 2003) and 906 µg/L in Muzaffar Ghar district (Farooqi *et al.,* 2007).

Over all concentration of arsenic was found highest in the meat samples of Peshawar city because of industrialization and urbanization but still the level of arsenic in meat is not much harmful for human consumption. Higher concentration of arsenic in the livers of cattle and goats has also been reported by Krupa and Swida, 1997.

Arsenic concentration in chicken's liver samples was higher than muscle as estimated by Wallinga, 2006 and Ghosh *et al.*, 2012. Arsenic has been used in the chicken feed from the last 60 years (Ratnaike, 2003). Roxarsone, approved as the feed additive in 1944 by Food and Drug Administration has been banned due to harmful effects but still used in most of the countries including Pakistan (Environmental impact report, 1981). In the mutton samples high concentration of arsenic was found in muscle samples as compare to liver similar results were estimated by Sathyamoorthy *et al.,* 2016. In the beef samples high level of arsenic was in muscles and liver as results were found by Khalafalla *et al.,* 2011.

Arsenic is a poison that gradually stored in the liver, kidney, skin and hair of the body (Vreman *et al.,* 1986). Residual of arsenic in tissues of animals indicates the existence of arsenic in environment because of copper smelting, coal combustion, burning of firewood and cow dung (Charles and Margaret, 1993).

Many studies have been conducted on arsenic by researchers in Pakistan. Mariam *et al*, 2004 worked on chicken, mutton and beef according to the results liver and muscles samples of different meat types were above the permissible limits (table 4).

Table: 4 Concentration of arsenic in liver and muscle of meat in other regions of world.

Location	**Chicken**		**Mutton**		**Beef**		**Reference**
	Liver	**Muscles**	**Liver**	**Muscles**	**Liver**	**Muscles**	
Pakistan	54.14±50.65ppb	60.60±32.61ppb	115.8±67.02ppb	165.4±124.6ppb	137.4±24.37ppb	205.7±149.9ppb	Present study
USA	221.8 parts per billion	20.1 parts per billion	---	--	--	--	Wallinga, 2006.
Canada	--	3.1µg/kgs	–	--	--	--	Liu *et al.*, 2016
Pakistan	46.77 + 5.33ppm	44.09 + 3.62ppm	42.78 +3.80ppm	42.40 + 4.95ppm	52 .4 4 + 5.22ppm	46.46 + 3.41ppm	Mariam *et al.*, 2004
South India	-	-	-	3.7±1.1 mg/kg, 5.6±1.7 mg/kg	-	-	Sathyamoorthy *et al.*, 2016
Bangladesh	102.1 ± 8.0 µg/kg	67.8 ± 5.1 µg/kg	-	-	-	-	Ghosh *et al.*,2012
Nigeria	0.03±0.01 µg/g	-	0.34±0.23 µg/g	-	0.08±0.01 µg/g	-	Akan *et al.*, 2010
Egypt	-	-	-	-	4.64 ± 0.40 mg/kg	5.06 ± 0.46 mg/kg	Khalafalla *et al.*, 2011
Ghana	-	-	-	0.014 ±0.0020 mg/kg	-	0.007± 0.0005 mg/kg	Nkansah *et al.*, 2014
Nigeria	0.0033 ± 0.001 mg/g	0.0802 ± 0.021 mg/g	-	-	-	-	Mohammed *et al.*, 2013
Pakistan	0.30±0.25 ppm	0.59±0.58 ppm	-	-	-	-	Imran *et al.*, 2015
Spain	-	-	-	-	0.043, mg/kg	0.004mg/kg	Lopez *et al.*, 2000

The research conducted by Imran *et al*, 2015 found that arsenic concentration was higher in muscle as compare to liver (table 4) and under the permissible limits. The results of present study showed level of arsenic below permissible limits.

Many studies have been conducted to estimate the human health risk assessment through different mediums such as through hair, blood, nails samples and content of heavy metal in an object. Javed and Usmani in 2013 calculated the concentration of heavy metals such as Ni, Cu, Fe, Co, Mn in the rivulet water released by many industries in Aligarh. The concentration of heavy metals was above the permissible limits. For the assessment of human health risk Javed and his fellow used hair samples of the nearby area's population (Javed and Usmani, 2013). Currently in the world hazard index or human health risk is widely estimated through hair of human. The purpose of human health risk assessment is to calculate the environmental exposure of the toxic metal and its nutritional status for human (Wang *et al.*, 2009). In present study The Human Health Risk was assessed through the arsenic concentration in meat samples also estimated by (Zhaung *et al.*, 2014). Zhaung and his fellows used different medium such as vegetable, fish meat, grains and rice to assess the hazard index in human beings through different heavy metals. Current study showed high Human Health Risk by consuming liver samples of all selected cities, on daily basis (33.43, 39.59, 50.49, 65.87). The highest hazard index was found in the samples of Peshawar. It has been found that the industrialization, intentional use of arsenic in chicken feed and contaminated water are the major sources of risk due to meat consumption. Water quality in the vicinity of Peshawar is very low, arsenic and other heavy metals are present in drinking water, used for animals as well as for human beings. Meat sold in markets is full of contamination because of open display of it and people do not pay attention to the hygiene of the meat and other food products. Consumption of liver of the chicken, goat and cow is very harmful regarding to the arsenic concentration because the biotransformation process of arsenic held in liver. Sometimes small amount of arsenic left in liver which cause severe health effects on the animal as well as on the population who consumed it. Muscles of chicken, goat and cow also showed high risk to humans because when arsenic entered in the body, it spread in different organs, through liver this toxic metal release into the urine but from other organs it does not transformed and release into urine such as in muscles of the animal body so that consumption of muscle of the contaminated meat are also very hazardous for eating purpose. In Gujrat, brackish water is used for the irrigation purpose due to which arsenic entered into food chain and exposed to human and animals. Lahore is the city full of pollution. Main pollution comes through the traffic and industrialization. Main source of water in Lahore is through river ravi which is the most contaminated river in Pakistan because the waste of many industries and also domestic waste directly goes into the river ravi without any treatment. This contaminated water than used by animals and human and exposed to toxic metals like arsenic. A study conducted in Bangladesh showed significant amount of arsenic groundwater that used for animals and human drinking purposes. As compare to China, India and Bangladesh, Pakistan has low level of arsenic in drinking water some years before but now the level is increasing rapidly due to less attention pay by government and people (Ali *et al.*, 2013). As in 2025, expected increase in human population is from 180 million to 221 million, it depicts the decrease in fresh water availability in Pakistan for human and animals. The drop of fresh water will be 5600 m3 to 1000m3 per capita in the year 2025 (Ahmad *et al.,* 2013). In all the cities of Pakistan water quality is contaminating vastly. In Pakistan, screening of water as compare to other countries started late, in the year 2000 which is also the reason of increase in arsenic concentration (Pervaz and Akram, 2016). In Islamabad, contamination and risk to human through meat is less as compare to other cities. The reason behind is less pollution, greenery, and limited industrialization within the city, but still meat of chicken, goat and cow is posing some harmful effects on humans.

The concentration of arsenic in the meat samples was under the permissible limits but the daily exposure of arsenic through meat caused severe health risk for the human beings. Excessive amount of arsenic (>1000 ng/m3) is present in the territory of industrial sources which directly impact negatively on human beings (USNRC, 1999; Ball et al., 1983; WHO, 1987). The residual of arsenic passes through the gastrointestinal tract absorbed in the body and then release via kidney (Buchet *et al.,* 1981; Luten *et al.,* 1982; Tam *et al.,* 1982). In human beings, half life of arsenic is between 2-40 days and it accumulates in the liver, kidney, skin, bone and muscles (Pomroy *et al.,* 1980; Ishinishi *et al.,* 1986).

Concentration of arsenic in chicken samples from all the cities (fig 1) is not harmful enough to consume but small amount of arsenic should also be avoided. Similar results were recorded by (Anderson and Chamblee, 2001; Nachman *et al.,* 2013; Imran *et al.,* 2015). According to EPA chicken consist of arsenic, causes many diseases, most prominently different types of cancer such as bladder, kidney, lungs, liver and prostate (Fact sheet, EPA). Arsenic exposure is also associated with the risk of cardiovascular disease neurological diseases in children and diabetes (Silbergeld and Nachman, 2008). Lung cancer is mostly found in the people live near the arsenic releasing industries (DEFRA, 2002). One another major effect of arsenic is on the human bladder and thio-arsenicals sometimes present in the human urine which are highly toxic for health (Sing *et al.,* 2015). It was observed from different studies that arsenic capability to interrupt the hormonal function among cells from mammals exposed to arsenic toxicity below than the level suggested by EPA (10ppb) (Mead, 2005; Bodwell *et al.,* 2004).

Mutton and beef samples also showed concentration of arsenic which was below the permissible limits (0.5 ppm). Similar results were found by (Bertin *et al.,* 2013). Red meat consists of arsenic when ingested, may cause severe cardiovascular system's disorder. Arsenic exposure both acute and chronic causes heart failure (Fennel and stacy, 1981; Goldsmith and from, 1986; Baykov et al., 2008) and sometimes causes hyperpigmentation in skin, skin nodules, vessel disease and even fatality due to high blood pressure (Fennel and stacy, 1981).

In year 2000, a study conducted by Hopenhyne-Rich and his fellows revealed that neonatal and postnatal mortality of infants were occurred in Chile due to exposure of arsenic to the pregnant women (Hopenhyne-Rich *et al.,* 2000). Milton and colleagues estimated the risk of stillbirth in female category due to the arsenic level of 100µg/L (Milton *et al.,* 2005). Many stillbirth cases are also present in Pakistan due to exposure of arsenic via foodstuff. Another study held by Ahmad *et al.,* 2005 showed the high rate of spontaneous abortions, stillbirth and premature deliveries in the females due to exposure to arsenic.

When people exposed to arsenic for a long period of time, peripheral nervous symptoms start appearing, estimated by an epidemiological study conducted by (Yang and Liang, 2003). The production of NO and reactive oxygen in the body has been increases 20-25% but DNA and protein synthesis decreases by 62% and 54%. So in this way arsenic can also cause damage to the brain and bones of the body (Chattopadhyay *et al.*, 2002). Aptosis, neutral networking, growth of viable cells was observed due to exposure of brain cells to arsenic for approximately 24 hours. When a person exposed to arsenic for 18 days, it may causes neurosis and loss of matrix. Necrosis and loss of ground matrix was observed after 18 days of arsenic exposure (Chattopadhyay *et al.*, 2002).

An arsenic based study conducted in west Bengal and Bangladesh showed the high rate of cough, shortness of breath and chest sound in lungs of male and female categories. As age and exposure to arsenic increases the health impacts increases also. (Sing *et al.*, 2008). In Pakistan, by understanding the pathways of arsenic exposure to humans, scientist can generate more studies for the assessment of arsenic toxicity through meat consumed in the whole country.

Chapter # 6

CONCLUSION AND RECOMMENDATIONS

CONCLUSION

This research confirmed the presence of arsenic residuals in the liver and muscle samples of chicken, mutton and beef in the selected sites. The arsenic accumulation was equally present in liver and muscle. The excessive increase in the industrialization and urbanization is the main reason of arsenic in the meat. Some natural exposures are also the reason behind. Although, arsenic contamination was below the permissible limits, set by ANZFA but still their consumption for human beings is harmful. Weak and malnutritious population is more vulnerable to it. The concerned authorities and organizations should take serious notice to minimize the arsenic content present in meat as it poses serious health effects on humans. The standard of arsenic residuals in drinking water has been reduced by reviewing it but the standard for arsenic in animal's organ is remain unchanged and need to be addressed again. Lack of knowledge and attention on the harmful effects of arsenic via meat, becomes the reason of increase in the hazards linked with it.

RECOMMENDATIONS

Keeping in view the results of present study, it is recommended that:

1. Ongoing and continuous monitoring of arsenic contamination is important as it build up the toxic concentration.
2. Arsenic contamination should be decreased by applying limits on the intentional use in the feed of chickens.
3. Water used for the drinking purpose of animals should not extract from the arsenic rich tube wells, or tube wells should not dig on the arsenic contaminated areas.
4. Campaigns and workshops should be organized to spread awareness of arsenic toxicity among people.
5. On the work places, workers should provide with the personal protection equipments so that no harmful effects will occur due to accidental splashes of arsenic acids.
6. Fewer amounts of broilers should be used for the consumption because they have arsenicals in their feed.
7. The plants that animals used for eating purposes, should not treated with the arsenic containing pesticides.
8. More research is needed to determine the effects of arsenic on human beings and animals.
9. As meat is an important diet for human beings so it should be free of any heavy metal and consumer should buy fresh and covered meat from the market.

10. Organizations should also ensure the hygienic measures in the slaughter houses and poultry farms and proper suitable environment should be provided to the animals.
11. More laboratories should be established in Pakistan to test and treat the arsenic containing food and should find more ways to cure the diseases occurred due to it.

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