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DETERMINATION OF HEAVY METALS IN RAW MILK SAMPLES FROM DIFFERENT AREAS OF LAHORE

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Abstract:

Study was conducted to analyze the concentration of different heavy metals including lead (Pb), Chromium (Cr), Zinc (Zn), Nickel (Ni), Manganese (Mn), Copper (Cu) and Iron (Fe) in raw milk samples from four towns of Lahore (Ravi Town, Data Gang Baksh Town, Iqbal Town and Town ship). 5ml of each sample was digested by using 14 ml HNO_3 and after 30 minutes by adding 10 ml $HCL0_4$ for almost 2 hours at the temperature of 250° C until vine green or color less solution is formed. The concentration of heavy metals were determined by using Atomic Absorption Spectrophotometer. The mean concentration of all the heavy metals in all these towns except Zn and Fe were above the permissible limit set by WHO. The concentration of Mn and Ni was slightly high. Heavy metals having highest toxicity have higher contamination and lowest toxicity has lowest contamination. Highest toxic metal Pb (WHO standard is 0.02 ppm) contamination ranges between 3.3 ppm to 6.3 ppm among these towns. The lowest toxic heavy metal Zn (WHO standard is 60 ppm) having highest nutritious value, the contamination ranges lie between 1.41 ppm to 3.6 ppm. The results shows that milk in Lahore has little to low nutritious value with high contamination. According to analysis it was found that the contamination of Township milk samples in heavy metals (Cr, Fe and Zn) was comparatively high as compared to other towns. The contamination of all the selected heavy metals in milk samples of Data Gang Baksh Town were comparatively low.

Keywords: Milk, Heavy metals, Contamination, Low nutrition, Lahore

INTRODUCTION:

Heavy metals are present naturally in the environment (Albert 1950). The increase in urbanization and industrialization the concentration of heavy metals has alarmingly increased which lead to cause health issues. Common sources of these heavy metals are industrialization, mining, vehicular emissions, increase in solid waste and improper solid waste disposal (Howell *et al*., 2012). Heavy metals such as lead and mercury enter in the environment through these sources and bio accumulates and bio magnify in the food chain (Pezzarossa *et al*., 2011). The increase of these heavy metals in the environment has caused lots of health problems some of the major diseases includes Lung inflammation, Cancer, pulmonary fibrosis, Nephrophaty, Pink disease, Brain damage, Tremor and Stomatitis (Nielen *et al*., 2008).

According to the recent studies heavy metals have been found in almost every consumable thing present in the environment due to the increase in urbanization and industrialization. They have been found in water, plants, fruits, milk and vegetables (Alloy, 2012). The tendency of bioaccumulation and bio magnification has not only caused health problems but also caused the degradation of food products. One of the most affected food product is milk.

Milk is one of the most important and beneficial food product globally. It is the complete diet in itself. Its significance is evident from the fact that the infants only consume milk in the early stages of life. It is the complete diet in itself (Dror *et al*., 2011) .Milk contains many nutrients including calcium, phosphorous, magnesium and potassium. All of these nutrients play an important role in proper functioning and building of body. Calcium is absorbed readily by the body which helps in the formation of bones. Phosphorous helps the body to utilize and absorb calcium and should be in proper proportion to calcium for the formation of bones. Milk has the ability to provide these two important minerals in the same quantity needed by the body to form bones. It also contains a significant source of riboflavin (vitamin B12) vitamin A and D which play an important role to improve the health of eyes and skin (Fox *et al*., 1999).

In Pakistan fields are irrigated by industrial waste water which contains high amount of heavy metals like Chromium, Copper, Lead, Arsenic, Iron, Nickel, and Zinc. These heavy metals have the ability to bio accumulate in the food chain and causes lots of damaging health effects to the consumers (Hussain *et al*., 2002). Some of these heavy metals are important for the normal functioning of the body like zinc but long term exposure badly affects the health life of consumers (Pb, Cr).

The absorption of lead causes blood pressure, cardiovascular diseases, liver, and kidney dis-functioning and also reduces cognitive and intellectual performances in children. Nickel helps the body to produce red blood cells but the long term exposure causes skin irritation, heart and liver damage and weight loss. It is found in aquatic life but do not magnify in the food chain. Iron affects the liver, kidney and cardiovascular systems. Chromium is important mineral as it stimulates the activities of insulin and controls blood cholesterol levels in the body. Low level intake of chromium causes skin irritation and ulcers. Long term causes kidney, liver, heart and tissue problems. Exposure of Copper causes Wilson disease, vomiting, diarrhea, hypertension and cardiovascular diseases. Manganese is an essential nutrient but high concentrations cause mental and emotional disturbances, male infertility, birth defects, neurological and neuromuscular disorders. Zinc exposure causes anemia, Leucopenia, gastrointestinal diseases and diarrhea (Ogabiela *et al.*, 2011).

Due to these reasons WHO sets the guidelines for these heavy metals in milk and milk products. The permissible value for Copper is 0.01 ppm. Lead is 0.02 ppm, Zinc is 60ppm, Chromium is 50-200 µg/day, Manganese 2.5mg/kg for adults and 20 µg for infants, Iron is 15mg (WHO, 1973).

The aim of this study was to check the heavy metals contamination in raw milk in 4 different towns of Lahore which includes Township, Ravi Town, Iqbal Town, and Data Gang Baksh town and to compare them with the standards (WHO, 1973).

MATERIALS AND METHODS

The study was conducted at Department of Environmental Sciences, University of Veterinary and Animal Sciences, Lahore.

Sample Collection:

The milk samples was taken from the 4 towns of Lahore from the recognized shops of the following selected towns.

1. Township
2. Ravi Town
3. Data Gang Baksh Town
4. Iqbal Town

Milk samples were collected from 5 different shops of each town. 6 samples were collected from each shop.120 samples were analyzed.

Quantitative Method for Heavy metals:

Digestion:

Take 5 ml sample and add 14 ml HNO_3 in a kjeldhals flask. After 30 minutes add 10 ml $HClO_4$. Heat the flask at 150 C after 15 min. Then heat it again at 250 C after 30 minutes. Until Vine green color or colour less solution is formed.

Then make the final volume up to 25 ml.

Heavy Metals Detection:

The milk was analyzed for heavy metals like Pb, Cu, Zn, Cr, Mn Ni and Fe by atomic absorption spectrophotometer (Sapumohotii *et al.,* 2015). The digested samples were measured using Atomic Absorption Spectrophotometer **(Polarized Zeeman Z-8230 Atomic Absorption Spectrophotometer)** after calibrating the instrument for each metal by feeding respective standards.

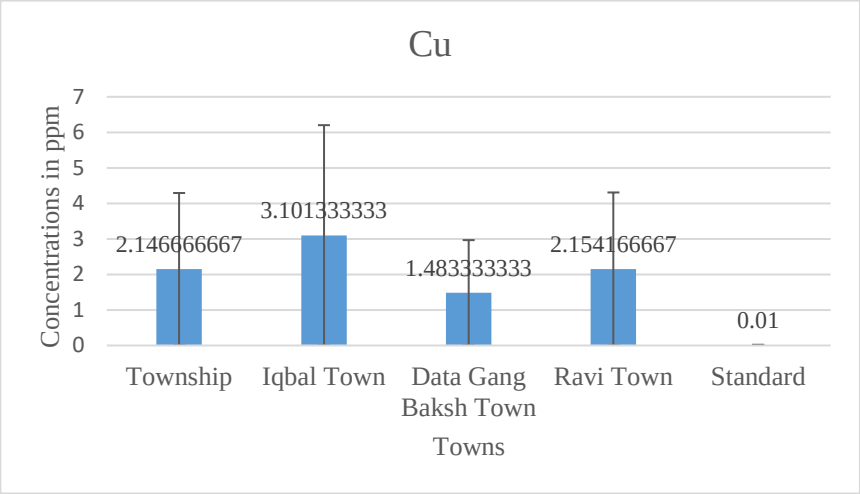
Statistical tools:

Standard Deviation was applied as a statistical tool. One way Annona was used to determine the statistical significance and variance for different treatments in SPSS software

RESULTS

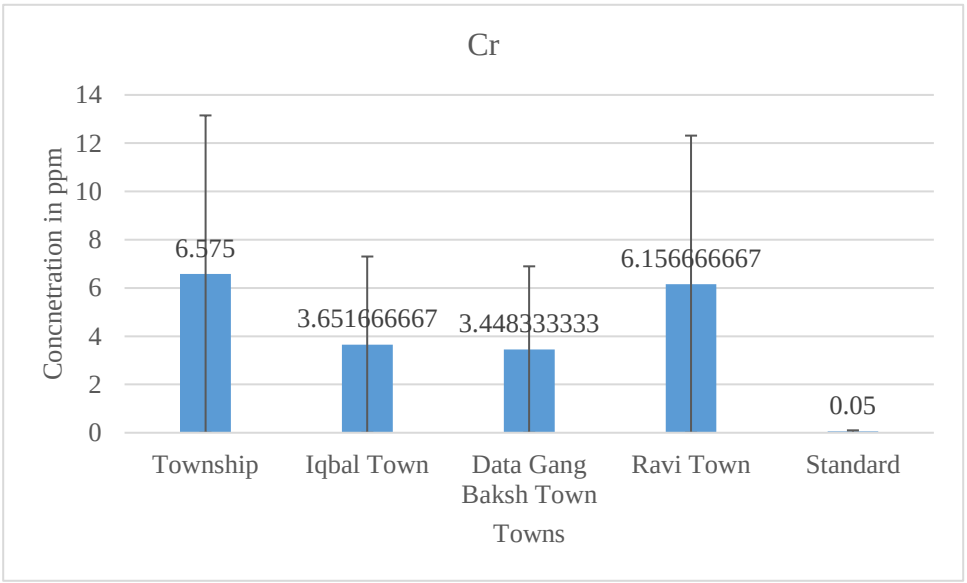
The raw milk samples were collected from different areas of Lahore. Four towns were selected Township, Iqbal Town, Datta Gang Baksh Town (DGB) and Ravi town. Samples were collected from the recognized shops of these areas. Samples were divided into these respective towns. Samples were digested by di acid method. After digestion, samples were analyzed by atomic absorption spectrophotometer, for heavy metals as follows: Pb (Lead), Cu (Copper), Zn (Zinc), Cr (Chromium), Mn (Manganese), Ni (Nickel), Fe (Iron). Results are discussed in this section.

Fig 1: Concentration of Cu



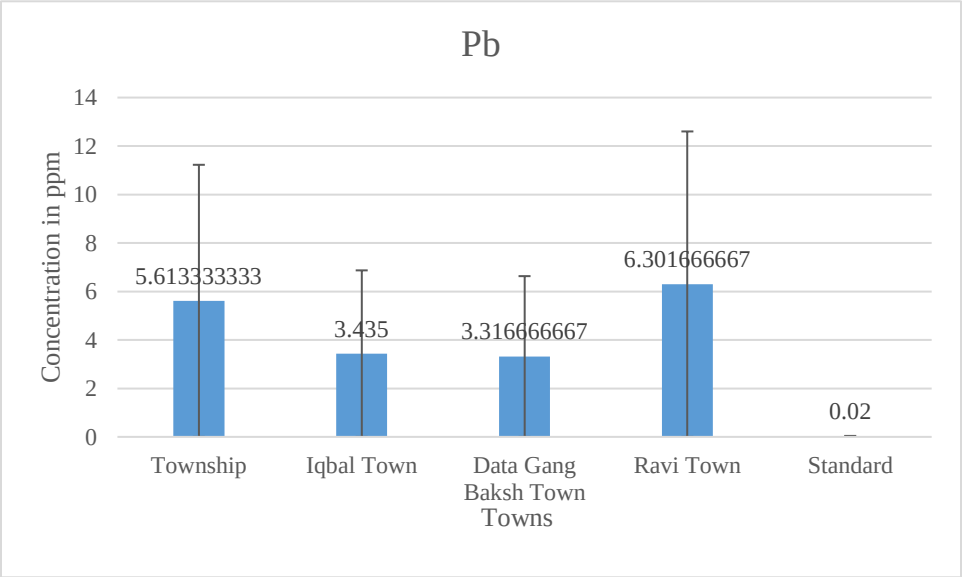
After analysis it was found that the concentration of copper was above the permissible limit in all these towns. Raw milk samples of Iqbal Town shows the maximum concentration. The standard for Cu is 0.01 ppm while mean concentrations in Iqbal Town was 3.10 ppm and lowest concentration was found in Data Gang Baksh Town having 1.48 ppm. The results in case of Cu are non-significant.

Fig 2: Concentration of Cr



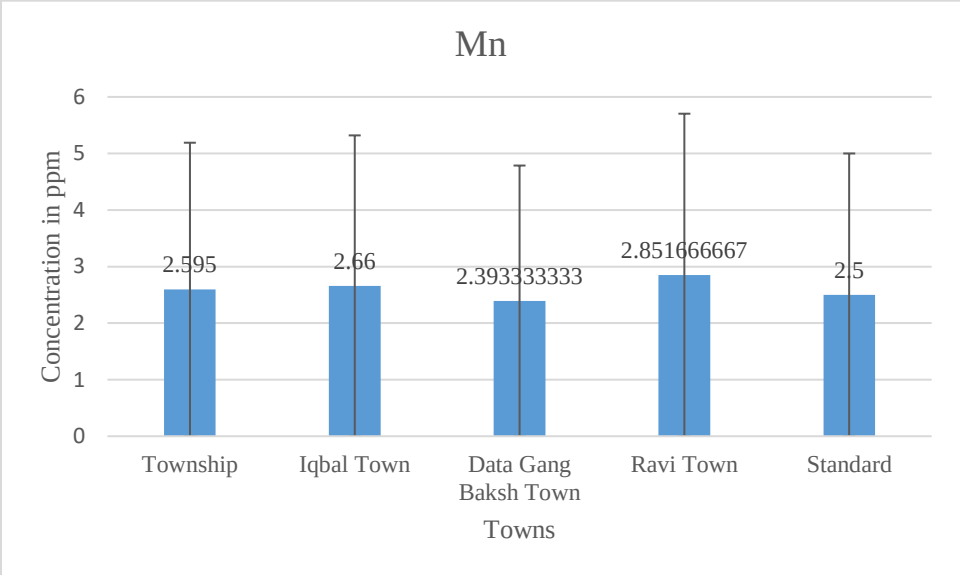
Milk Samples of Township has highest concentration of Cr. The concentration of Cr is 6.577 ppm. The lowest concentration was found in Data Gang Baksh Town with 3.48 ppm. It was found that the results are significant after applying stats.

Fig 3: Concentration of Pb.



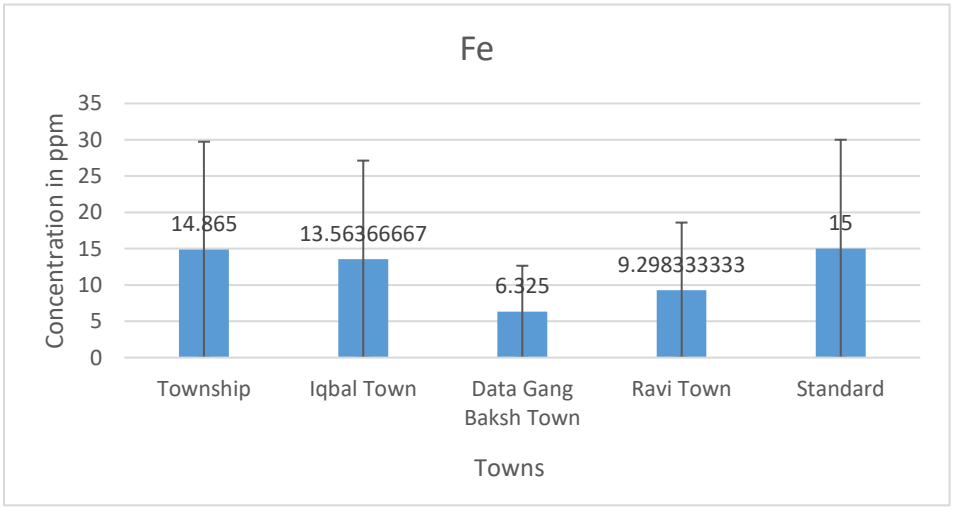
The highest concentration of Pb was found in Ravi Town milk samples; 6.30 ppm. The lowest concentration was 3.31, found in Data Gang Baksh Town. The concentrations were above the permissible limit in all these towns, which shows that milk is contaminated. The results shows that milk is contaminated in terms of Pb in all these towns. The results were significant as p value was greater than 0.05.

Fig 4: Concentration of Mn



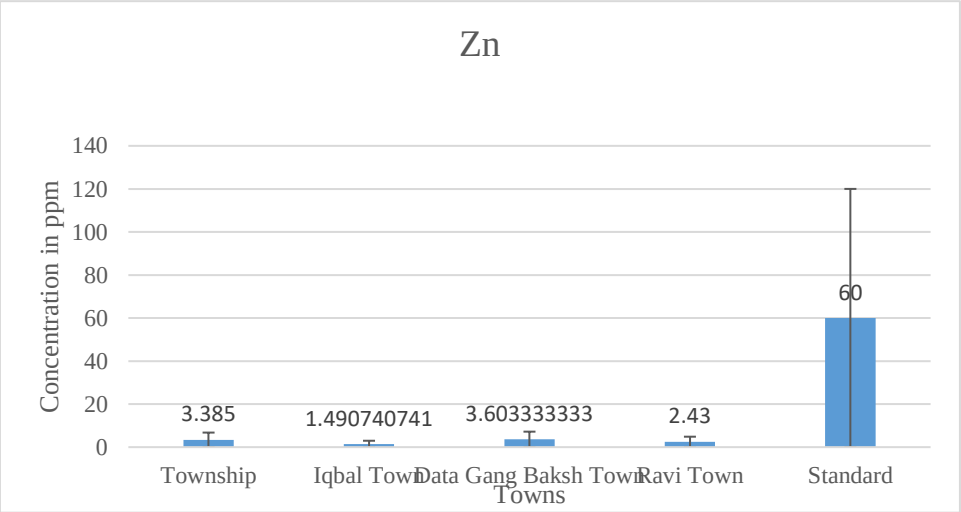
The concentration of Mn was high in Ravi town milk samples (2.85 ppm). Data Gang Baksh Town milk samples has lowest concentration of Mn (2.39 ppm). The concentration of this town was below the permissible limit which shows that milk is consumable in terms of Mn. The milk samples for rest of the town is contaminated.

Fig 5: Concentration of Fe



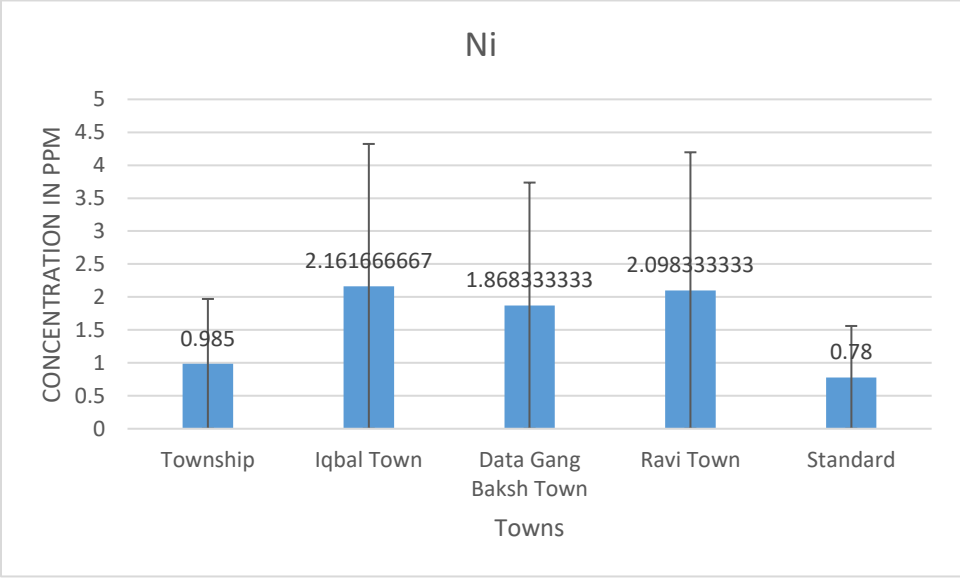
Fe is one of the most metal. All the concentration in all these towns was below the permissible limit. Highest concentration was found in township milk samples (14.365 ppm). Township milk samples contain high nutritional levels in terms of Fe. DGB contains lowest Fe concentration this shows having low nutritional value.

Fig 6: Concentration of Zn



The concentration of Zn was below the permissible limit in all milk samples. The concentration of Zn was highest in DGB (3.60 ppm) and lowest in Iqbal Town milk (1.40 ppm). The lowest concentration shows in all these towns shows that milk do not contain adequate nutritional value.

Fig 7: Concentration of Ni



The concentration of Ni was above the permissible limit in all milk samples. The concentration of Zn was highest in Iqbal Town (2.16 ppm) and lowest in Township milk (0.95 ppm). The concentrations in all these towns was slightly above the permissible limit (WHO, 1973).

DISCUSSION

Four towns were selected which includes Township, Ravi Town, Datta Gang Baksh Town and Iqbal town. Five famous shops were selected. 10 samples were collected from each town. Total 120 samples were collected including replications. These samples were then digested on the digestion blog and heavy metals were determined by using Atomic Absorption Spectrophotometer.

Concentration of heavy metals in Township Milk sample

Chromium (Cr):

Chromium is essential for human health, the high concentration can cause lots of disease as discussed in previous chapters.

In the present study the mean value for chromium was 15.285 ppm in Township milk sample while the permissible limit for Cr according to WHO was found 0.05 ppm. A study was conducted to check the heavy metals in different areas of Lahore to analyze the milk samples. Chromium concentrations were high in different areas of Lahore (Naeem *et al*., 2015).

Lead (Pb):

Lead is important in the normal functioning of human body but the increase in concentration due to urbanization and industrialization results in the increase in concentration which can cause lot of diseases.

In the present study the mean value for lead is 13.066 ppm in Township while the permissible limit for Lead is 0.02 ppm according to WHO. Study was conducted in different areas of Iran to determine the concentration of Lead in milk samples. The concentration of lead was above the permissible value (Derakhshesh and Rahimi, 2012).

Copper (Cu):

Copper is one of the most essential nutrient for the human body but the high concentration can cause lots of problem in the environment as well as on the human body.

The mean concentration of Cu in the Township milk sample are 4.972 ppm while the standard of World Health Organization (WHO) for Cu is 0.01 ppm. Toxic metals like copper was determined in milk samples of different areas of Iran. The concnentration of Copper in the milk samples were above the permissible limit (Derakhshesh and Rahimi, 2012).

Zinc (Zn):

Zinc is important for the normal functioning of human body and the toxicity is less harmful as compared to other metals.

The mean value in the present study of zinc in township samples are 7.778 ppm while the permissible limit of World Health Organization (WHO) for Zn is 60 ppm. Study was conducted to check the concentration of heavy metals such as zinc in milk samples of different areas of Nigeria. The concentration of zinc were below the permissible limit (Ogabiela *et al*., 2011).

Manganese (Mn):

Manganese causes lots of problems in the environment as well as on human body if the concentration increases in the environment.

The mean value in the present study of manganese in township samples is 6.9 ppm while the permissible limit of World Health Organization (WHO) is 2.5 ppm. Investigation was conducted to determine the concentration of heavy metals such as manganese in different areas of Ethopia. The concentration of manganese was above the permissible limit (Belete *et al*., 2012).

Nickel (Ni):

Researches are still going on to study the mechanism of its effect on human body. In the present study the mean value of Ni in Township is 2.241 ppm while the standard of World Health Organization (WHO) for Ni is 0.75

ppm. The study was conducted to investigate the nickel concentration in different areas of KPK, Pakistan. The concentration of nickel were above the permissible limit (Kazi *et al*., 2009).

Iron (Fe):

Iron is one of the most essential heavy metal. The concentration of this heavy metal above the permissible limit can cause lots of problems. Studies are still going on to study its toxic mechanism on human body.

The mean value of iron in township milk sample is 34.485 ppm while the standard of World Health Organization (WHO) for Fe is 15 ppm. Study was conducted to check the heavy metal concentration such as Fe in milk samples of Egypt. The concentration was high above the permissible limit (Malhat *et al*., 2012).

Concentration of heavy metals in Iqbal Town Milk sample

Chromium (Cr):

Chromium is essential for human health, the high concentration can cause lots of disease as discussed in previous chapters.

In the present study the mean value for chromium was 8.524 ppm in Iqbal Town milk sample while the permissible limit for Cr according to WHO was found 0.05 ppm. A study was conducted to check the heavy metals in different areas of Lahore to analyze the milk samples. Chromium concentrations were high in different areas of Lahore (Naeem *et al*., 2015).

Lead (Pb):

Lead is important in the normal functioning of human body but the increase in concentration due to urbanization and industrialization results in the increase in concentration which can cause lot of diseases.

In the present study the mean value for lead is 7.909 ppm in Iqbal Town while the permissible limit for Lead is 0.02 ppm according to WHO. Study was conducted in different areas of Iran to determine the concentration of Lead in milk samples. The concentration of lead was above the permissible value (Derakhshesh and Rahimi, 2012).

Copper (Cu):

Copper is one of the most essential nutrient for the human body but the high concentration can cause lots of problem in the environment as well as on the human body.

The mean concentration of Cu in the Iqbal Town milk sample are 7.403 ppm while the standard of World Health Organization (WHO) for Cu is 0.01 ppm. Toxic metals like copper was determined in milk samples of different areas of Iran. The concnentration of Copper in the milk samples were above the permissible limit (Derakhshesh and Rahimi, 2012).

Zinc (Zn):

Zinc is important for the normal functioning of human body and the toxicity is less harmful as compared to other metals.

The mean value in the present study of zinc in Iqbal Town milk samples are 3.53 ppm while the permissible limit of World Health Organization (WHO) for Zn is 60 ppm. Study was conducted to check the concentration of heavy metals such as zinc in milk samples of different areas of Nigeria. The concentration of zinc were below the permissible limit (Ogabiela *et al*., 2011).

Manganese (Mn):

Manganese causes lots of problems in the environment as well as on human body if the concentration increases in the environment.

The mean value in the present study of manganese in Iqbal Town milk samples is 6.132 ppm while the permissible limit of World Health Organization (WHO) is 2.5 ppm. Investigation was conducted to determine the concentration of heavy metals such as manganese in different areas of Ethopia. The concentration of manganese was above the permissible limit (Belete *et al*., 2012).

Nickel (Ni):

Researches are still going on to study the mechanism of its effect on human body. In the present study the mean value of Ni in Iqbal Town is 5.017 ppm while the standard of World Health Organization (WHO) for Ni is 0.75 ppm. The study was conducted to investigate the nickel concentration in different areas of KPK, Pakistan. The concentration of nickel were above the permissible limit (Kazi *et al*., 2009).

Iron (Fe):

Iron is one of the most essential heavy metal. The concentration of this heavy metal above the permissible limit can cause lots of problems. Studies are still going on to study its toxic mechanism on human body.

The mean value of iron in Iqbal Town milk sample is 34.285 ppm while the standard of World Health Organization (WHO) for Fe is 15 ppm. Study was conducted to check the heavy metal concentration such as Fe in milk samples of Egypt. The concentration was high above the permissible limit (Malhat *et al*., 2012).

Concentration of heavy metals in Datta Gang Baksh Town Milk sample

Chromium (Cr):

Chromium is essential for human health, the high concentration can cause lots of disease as discussed in previous chapters.

In the present study the mean value for chromium was 7.973 ppm in Datta Gang Baksh Town milk sample while the permissible limit for Cr according to WHO was found 0.05 ppm. A study was conducted to check the heavy metals in different areas of Lahore to analyze the milk samples. Chromium concentrations were high in different areas of Lahore (Naeem *et al*., 2015).

Lead (Pb):

Lead is important in the normal functioning of human body but the increase in concentration due to urbanization and industrialization results in the increase in concentration which can cause lot of diseases.

In the present study the mean value for lead is 7.61 ppm in Datta Gang Baksh Town while the permissible limit for Lead is 0.02 ppm according to WHO. Study was conducted in different areas of Iran to determine the concentration of Lead in milk samples. The concentration of lead was above the permissible value (Derakhshesh and Rahimi, 2012).

Copper (Cu):

Copper is one of the most essential nutrient for the human body but the high concentration can cause lots of problem in the environment as well as on the human body.

The mean concentration of Cu in the Datta Gang Baksh milk sample are 7.403 ppm while the standard of World Health Organization (WHO) for Cu is 0.01 ppm. Toxic metals like copper was determined in milk samples of different areas of Iran. The concentration of Copper in the milk samples were above the permissible limit (Derakhshesh and Rahimi, 2012).

Zinc (Zn):

Zinc is important for the normal functioning of human body and the toxicity is less harmful as compared to other metals.

The mean value in the present study of zinc in Datta Gang Baksh Town milk samples are 8.44 ppm while the permissible limit of World Health Organization (WHO) for Zn is 60 ppm. Study was conducted to check the concentration of heavy metals such as zinc in milk samples of different areas of Nigeria. The concentration of zinc were below the permissible limit (Ogabiela *et al*., 2011).

Manganese (Mn):

Manganese causes lots of problems in the environment as well as on human body if the concentration increases in the environment.

The mean value in the present study of manganese in Datta Gang Baksh Town milk samples is 5.5 ppm while the permissible limit of World Health Organization (WHO) is 2.5 ppm. Investigation was conducted to determine the concentration of heavy metals such as manganese in different areas of Ethopia. The concentration of manganese was above the permissible limit (Belete *et al*., 2012).

Nickel (Ni):

Researches are still going on to study the mechanism of its effect on human body. In the present study the mean value of Ni in Datta Gang Baksh Town is 4.28 ppm while the standard of World Health Organization (WHO) for Ni is 0.75 ppm. The study was conducted to investigate the nickel concentration in different areas of KPK, Pakistan. The concentration of nickel were above the permissible limit (Kazi *et al*., 2009).

Iron (Fe):

Iron is one of the most essential heavy metal. The concentration of this heavy metal above the permissible limit can cause lots of problems. Studies are still going on to study its toxic mechanism on human body.

The mean value of iron in Datta Gang Baksh Town milk sample is 14.71 ppm while the standard of World Health Organization (WHO) for Fe is 15 ppm. Study was conducted to check the heavy metal concentration such as Fe in milk samples of Egypt. The concentration was high above the permissible limit (Malhat *et al*., 2012).

Concentration of heavy metals in Ravi Town Milk sample

Chromium (Cr):

Chromium is essential for human health, the high concentration can cause lots of disease as discussed in previous chapters.

In the present study the mean value for chromium was 14.11 ppm in Ravi Town milk sample while the permissible limit for Cr according to WHO was found 0.05 ppm. A study was conducted to check the heavy metals in different areas of Lahore to analyze the milk samples. Chromium concentrations were high in different areas of Lahore (Naeem *et al*., 2015).

Lead (Pb):

Lead is important in the normal functioning of human body but the increase in concentration due to urbanization and industrialization results in the increase in concentration which can cause lot of diseases.

In the present study the mean value for lead is 14.55 ppm in Town while the permissible limit for Lead is 0.02 ppm according to WHO. Study was conducted in different areas of Iran to determine the concentration of Lead in milk samples. The concentration of lead was above the permissible value (Derakhshesh and Rahimi, 2012).

Copper (Cu):

Copper is one of the most essential nutrient for the human body but the high concentration can cause lots of problem in the environment as well as on the human body.

The mean concentration of Cu in the Ravi Town milk sample are 3.96 ppm while the standard of World Health Organization (WHO) for Cu is 0.01 ppm. Toxic metals like copper was determined in milk samples of different areas of Iran. The concnentration of Copper in the milk samples were above the permissible limit (Derakhshesh and Rahimi, 2012).

Zinc (Zn):

Zinc is important for the normal functioning of human body and the toxicity is less harmful as compared to other metals.

The mean value in the present study of zinc in Ravi Town milk samples are 3.33 ppm while the permissible limit of World Health Organization (WHO) for Zn is 60 ppm. Study was conducted to check the concentration of heavy metals such as zinc in milk samples of different areas of Nigeria. The concentration of zinc were below the permissible limit (Ogabiela *et al*., 2011).

Manganese (Mn):

Manganese causes lots of problems in the environment as well as on human body if the concentration increases in the environment.

The mean value in the present study of manganese in Ravi Town milk samples is 6.525 ppm while the permissible limit of World Health Organization (WHO) is 2.5 ppm. Investigation was conducted to determine the concentration of heavy metals such as manganese in different areas of Ethopia. The concentration of manganese was above the permissible limit (Belete *et al*., 2012).

Nickel (Ni):

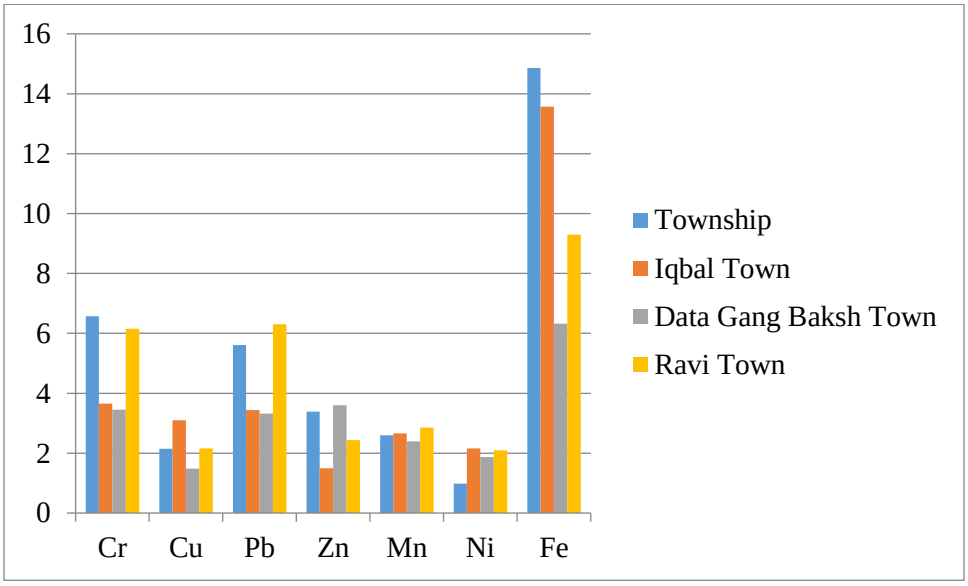
Researches are still going on to study the mechanism of its effect on human body. In the present study the mean value of Ni in Ravi Town is 4.17 ppm while the standard of World Health Organization (WHO) for Ni is 0.75 ppm. The study was conducted to investigate the nickel concentration in different areas of KPK, Pakistan. The concentration of nickel were above the permissible limit (Kazi *et al*., 2009).

Iron (Fe):

Iron is one of the most essential heavy metal. The concentration of this heavy metal above the permissible limit can cause lots of problems. Studies are still going on to study its toxic mechanism on human body.

The mean value of iron in Ravi Town milk sample is 20.68 ppm while the standard of World Health Organization (WHO) for Fe is 15 ppm. Study was conducted to check the heavy metal concentration such as Fe in milk samples of Egypt. The concentration was high above the permissible limit (Malhat *et al*., 2012).

Comparison of heavy metals between Towns



Bar Charts indicate the concentration of heavy metals of a particular town. According to this data, it is clear that the milk in Township is more contaminated as compared to other towns. All the selected heavy metals except Ni, Pb and Cu are in higher concentration than all other towns. In case of Cu and Ni, the concentration in Iqbal Town milk samples is higher than all the other towns. In case of Pb, the concentration of heavy metals in Ravi Town milk samples is comparatively high. Ni and Cu are not hazardous as compared to the other heavy metals like Cr. So it is concluded that water, fodder being utilized by the animals whose milk is being supplied to township is highly contaminated. The milk samples of DGB town is consumable as compared to other towns.

ANOVA was applied. The results of Ni and Cr were significant while the results of Mn, Cu, Fe, Zn and Pb were non-significant.

The results shows that milk is highly contaminated which can cause lots of diseases so regulatory authorities should work on the establishment of rules and regulations to cope with this issue

There are some recommendations which can be beneficial to minimize the heavy metal contaminants in milk sources:

- Provide clean and pure environment to animals like buffaloes, goats.
- They should be fed on clean and pure water and food.
- Animal sheds should be cleaned and washed at regular basis.
- Provide pure and healthy food and water.
- Water contamination should be properly checked.
- Avoid unnecessary use of medicines.
- Contact proper veterinary doctor if any animal looks diseased or behaves abnormal.
- The transportation of milk should be in insulated drums that are not coated with any harmful chemical or metal to avoid any possible reaction with metal.
- Animals should be dealt carefully and should be observed regularly to spot any abnormality.
- Animals should be vaccinated on time and by proper staff.

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