ANALYSIS OF TOXIC HEAVY METALS AND PESTICIDES IN DAIRY MILK OF PESHAWAR DISTRICT

By

DR. BUSHRA IFTIKHAR
PhD Scholar

Supervised By

PROF. DR. S. SHAFIQ-UR-REHMAN
Professor

DEPARTMENT OF ENVIRONMENTAL SCIENCES

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By Bushra Iftikhar

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Approved by

Principal Supervisor: _____

Prof. Dr. S. Shafiq-ur-Rahman
Department of Environmental Sciences
University of Peshawar

Co-Supervisor: _____

Dr. Samina Siddiqui
Assistant Professor
National Centre of Excellence in Geology
University of Peshawar

Examinar: _____

Prof. Dr. Shafi Muhammad Nizamani
National Centre of Anaylitical Chemistry
Sindh University, Jamshoro

Chairperson: _____

Department of Environmental Sciences
University of Peshawar

Department of Environmental Sciences
University of Peshawar
Session 2005-2006

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ABSTRACT

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Environmental pollution is one of the most difficult challenges faced by mankind today. The one that directly affects human health is the food pollution which has its roots in soil and water. There are certain food items which are an essential part of our diet like wheat flour, salt, tea, milk, rice, water etc. Since milk is produced from cow's food, it will be contaminated by anything that the cattle eat. The most dangerous of these contaminants are those that cannot be removed by ordinary treatments and these include toxic heavy metals, pesticides, hormones, persistent organic pollutants (POPs, some drugs, etc.

Heavy metals and pesticides gain entry into dairy milk through the diet of the cattle or drinking water. Heavy metals and pesticides are known to produce long term toxic effects on human body as these substances are stored in various tissues and organs of body and show bioaccumulation. Therefore there is a dire need to ascertain whether toxic heavy metals and pesticides are present in dairy milk in this area. As such this research was designed with the objective to assess common toxic heavy metals like cadmium, chromium, copper, cobalt, lead and nickel and lipophilic pesticides like cypermethrin, chlorpyrifos, and prophenofos in dairy milk and in animal feed and compare them with their maximum residue limits (MRLs) and find correlation, if any.

It was a cross-sectional study carried out in Peshawar district. The dairy farms were selected randomly from 3 urban and 2 rural areas. A total of 70 milk samples were analyzed, out of which 50 were of raw milk (30 urban and 20 rural), 10 of pasteurized milk and 10 of dry milk. The procedure carried out for pesticides was that liquid extraction by QuEChERs method was done first and then Gas Chromatography - Mass Spectrometry was used at the end for detection. The procedure adopted for toxic heavy metals was wet digestion with nitric acid and HCl and heating at 200°C on a hot plate followed by analysis on Atomic absorption spectrometry.

All heavy metals were found to be significantly greater in the urban farm 1 than the other four rural and urban farms. The heavy metal content in milk was greater as compared to the maximum residue limits (MRLs) given by International Dairy Federation (IDF) Standards 1977 or World Health Organization's and Food and Agricultural Organization's Joint Expert Committee on Food Additives (JECFA) 1989, 1993. Results of the present study show that the mean content of Cd, Cr, Cu, Co, Ni and Pb were 86, 24, 12, 14, 63 and 89 times greater respectively in raw dairy milk than maximum residue limits (JECFA 1989, 1993) for the same elements in normal milk. When heavy metal content was determined in fresh, pasteurized and dry milk samples, the results showed that there was greater content of Cd and Co in pasteurized and dry milk than fresh milk whereas Cr, Cu, Ni and Pb were greater in fresh milk than pasteurized and dry milk. All the heavy metals were also found in all the animal diets (cotton khal, sugar cane khal, maize, wheat, wheat bran and mixed diet) in varying amounts but were within the MRLs except for Copper in cotton khal which was very high. Cotton khal had comparatively

highest concentration of all the elements except Cr and Co whereas wheat bran had lowest concentration of all elements except Co which was highest in it. Correlation studies showed a weak positive correlation between heavy metal concentration in animal diet and dairy milk.

Dairy milk was also analyzed for certain GC amenable lipophilic pesticides _ cypermethrin, chlorpyriphos, and profenophos, which are used on crops that are the major component of animal feeds. Urban farm 1 had higher concentrations of these pesticides as compared to all other farms. Cypermethrin levels were higher in all the farms followed by chlorpyrifos whereas the concentration of profenofos was very low in all farms. Cypermethrin was 2 to 4 folds greater in all milk samples except for milk sample from rural farm 5. Similarly, chlorpyriphos was 4 to 10 folds greater in milk samples from all farms except for urban farms 2 and 3. However there were no significant variations in the concentration level of cypermethrin of all the farms. Same was the situation for chlorpyrifos. There was a trend that significantly greater ($p<0.05$) content of cypermethin, chlorpyrifos and profenofos were noted in raw milk than in pasteurized and dry milk. The profenofos content was more or less the same in pasteurized and dry milk but cypermethrin and chlorpyrifos were comparatively higher in pasteurized milk than in dry milk. The concentration of cypermethrin and chlorpyrifos were higher than the maximum residue limits of 0.05 and 0.02 mg kg^{-1} respectively while that of profenofos was within MRL of 0.01 mg kg^{-1}. As regards animal feed all the three were detected but cypermethrin highest, chlorpyrifos lower and profenophos was detected in very low concentrations. There was not much difference in the concentration of cypermethrin in different types of dairy cattle feed and the same was true for chlorpyrifos. The pesticides concentrations were however lower than the MRLs given for animal feed.

The results suggest that the raised levels of toxic trace elements in milk could be due to non-covalent binding with milk proteins particularly the casein, whereas elevated levels of lipophilic pesticides in milk may be due to selective solubility in milk fat. Therefore the presence of both is a matter of concern and can be handled with long term measures of policy of adopting Good Agricultural Practices (GAP) in growing forage crops and promoting awareness among dairy farm owners/workers with respect to change and improvements in dairy farming technology.

Key Words:

Cypermethrin, chlorpyrifos, profenofos, maximum resiudue limits (MRLs), biomagnification, bioaccumulation, pesticides, toxic heavy metals, hazardous

Chapter 1

INTRODUCTION

1.0. INTRODUCTION

Environmental pollution impact on human health has remained a challenge for the developing world since industrial revolution. Increase in industrialization and urbanization accelerated the input of inorganic as well as organic contaminants in air, water and soil. Inhaltion of air borne pollutants, drinking contaminated water and eating food grown over contaminated soils may cause severe health risks among humans and farm animals. A significant portion of these contaminants remain within body whereas other insignificant portion is excreted from the body.

1.1. Significance of milk for humans

Certain food items are a regular part of human diet and are essential for growth and development. Milk is one such food item which is consumed through out the world extensively as such or in the form of various products like butter, cheese, whey, deserts, ice-cream etc. Resultingly milk has a direct bearing on human health. Milk composition depends on cow's food and consists of lactalbumin, lactoglobulin, fibrinogen, casein and water. Variation in the ingredient content in milk primarily depends on the type and daily intake of diet by cattle. Cattle fed on diet according to their body requirements usually produce good quality milk which is a very important source of proteins, fats, carbohydrates, vitamins and minerals particularly Ca, Cl, Co, Cr, Cu, F, I, K, Mg, Mn, Mo, Na, Ni, P, S, Se, V and Zn which are definitely required for skeletal development and other growth functions. Severe deficiency symptoms appear when these are present in low concentrations than their normal recommended value for growth and various functions of the body (Table 1.1.1). Deformed bones are commonly found in humans

with low levels of Ca and Mg. Iodine deficiency causes goiter whereas skin lesion appears when Zn is present at low concentration in the body. Similarly, lack of iron results in reduction of red blood cell counts, resulting in anemia. Like deficiency symptoms, toxicity symptoms also appear when essential micronutrients are present in concentrations greater than their normal requirement for the body structure and growth. Iodine present in greater concentration in the body causes an unbalance in the thyroid gland function. High content of Fe in the body results in diarrhea, seizures and stomach aches (Richard, 2001).

Heavy metal toxicity primarily depends on the dietary intake in human. When dietary intake of heavy metals from food reaches above 30%, it is unsuitable for humans (Lindsay, 1986). Cattle fed on diet grown over contaminated soil or irrigated with contaminated water produce contaminated milk. Some of the heavy metals like Cd and Hg are non essential for body growth and other metabolic processes (Roussel et al., 2000; Rolfes et al., 2008). When Cd and Hg content is more than their acceptable levels within human body it causes long term cumulative toxicological effects (Daniel and Edward 1995; Cunningum and Saigo 1997). JECFA (1982) reported that if 1.5 mg kg^{-1} day^{-1} of arsenic is ingested it may cause chronic arsenic toxicity in humans and 0.15 mg $kg^{-1}day^{-1}$ of arsenic can cause toxicity later in life. Nevertheless the mode of pathological and cumulative toxic effects of heavy metals is complex and variable within human body (Phipps, 1976).

Pasteurization or heat treatment provides a complete remedy to remove pathogens and biological contamination from milk. However, inorganic and organic contaminants such as heavy metals, pesticides, hormones, POPs and antibiotics are unlikely to be removed

through heating whereas they are considered to be more toxic than biological contaminants when present at high concentration in cattle diet. Eating contaminated food or drinking contaminated milk and dairy products may cause heart diseases, cardiovascular accidents, obesity, cancers, allergies, digestive problem, diabetes and asthma in human beings (Rietz, 2010).

Table 1.1.1. The content of some elements in human body (adapted from, McDowell, 2010).

S #	Elements	Concentration (g)
1	Oxygen	43000
2	Carbon	16000
3	Hydrogen	7000
4	Nitrogen	1800
5	Calcium	1000
6	Phosphorus	780
7	Sulfur	140
8	Potassium	140
9	Sodium	100
10	Chlorine	95
11	Magnesium	19
12	Silicon	18
13	Iron	4.2
14	Fluorine	2.6
15	Zinc	2.3
16	Bromide	0.20
17	Lead	0.12
18	Copper	0.072
19	Aluminium	0.061
20	Cadmium	0.05
21	Boron	<0.048
22	Barium	0.022
23	Tin	<0.017
24	Manganese	0.012
25	Iodine	0.013
26	Nickel	0.01
27	Chromium	<0.0018
28	Cobalt	0.0015

1.2. Sources of heavy metal contamination in milk

Nutrients enter into the food chain of human beings through diet. Milk is a complete diet for us. Milk contains all essential micronutrients that are necessary for our health. However, the nutritional value of milk primarily depends on the types of feed and secondarily on daily intake of dairy cattle. Dairy cattle fed on the feed according to their health requirements will be producing good quality milk. Unluckily, the diet given to the dairy cattle at the farm is prepared from agro-industrial by product such as wheat, rice or cotton straw, sugarcane plup, stovers and green forages. The latter feeds are found to be low in lingo-cellulose hence are deficient in carbohydrates, proteins, energy, essential micronutrients and vitamins (McDowell, 2003; Suttle, 2010). Therefore, dairy cattle at the farm are more vulnerable to essential micronutrient deficiency most probably because forages grown on soils are deficient in one or more of these micronutrients. Rafiq and Sultani (2004) in their classical review on livestock production in Pakistan reported that dairy cattle grazing in soils deficient in P, K, B and Zn were producing milk low in these micronutrients. Dairy cattle when grazing in soil near mining sites, agricultural fields irrigated with industrial and municipal wastewater or fed on forages grown on contaminated soils are usually producing milk high in one or more of heavy metals (Tables 1.2.1 & 1.2.2.). These heavy metals are considered to be toxic once they enter into the food chain of grazing livestock. Heavy metal is a term commonly used for metals, non metals and metalloids (Russell, 1978). Elements with atomic number greater than 26, atomic weight between 63.546 – 200.590, and density greater than 5 g/cm^3 (density at least 5 times greater than water, high density means high stability and as such

little metabolism in the body) are known as heavy metals (Connel and Miller, 1984; Kennish, 1992). Heavy metals have two important characteristics:-

1. Their strong attraction to biological tissues
2. Their slow elimination from the body, which make them particularly hazardous to human health.

Heavy metals gain entry into the milk through cattle fodder which in turn is contaminated through the following sources:

Soil

Heavy metals are present in minute quantity in soil naturally. Nevertheless they are present in high concentration in some parts of the world whereas found to be deficient in other parts of the world. Some elements are found to be essential for plant growth whereas others are non essential. Plants can accumulate elements in their non edible parts e.g the shrub camellia sinensis (tea) can accumulate high content of Al and Mn in their leaves (Pennington, 1988). Becium homblei and grasses such as agrostis and festuca accumulate Cu, Cd, Hg, Ni, Cr in their roots grown near mining soil (Bradshaw et al., 1965; Reilly, 1969). Volcanic soils are very fertile and intensely cultivated but often contain many toxic metals like Hg (Reilly et al. 1989).

Metals in sewage sludge

Sewage sludge is used as organic fertilizer over the last several years. Sewage sludge is enriched in organic matter (40 %) and contains 2.4 % of N and 1.3 % of PO_4 essential for plant nutrients. Sewage sludge also contains high content of toxic metals such as Cd, Cr, Hg, Ni, Pb and Zn (Mackenzie and Purves, 1975).

Metal in fertilizers

Phosphate fertilizer is essential for various crops and considered to be a source of Cd in soils (Williams and David, 1973)

Metals in agrochemicals

Several metals are used in fungicides and other agricultural chemicals, for example copper sulphate mixed with lime and water in Bordeaux mixture, organic mercurial compounds, lead arsenate is used as an insecticidal spray and organic arsenic as a herbicide (Ministry of Agriculture, 1982).

Metals in water

Wastewater from industries, mining sites or domestic or municipal sites add a considerable amount of toxic metals in rivers, lakes, and seas. When wastewater is used for irrigation purposes it may cause toxicity to soil and plants and become a source of groundwater contamination.

Metals in food manufacturing plants & utensils

Interactions can occur between various metallic structures used in industries making edible items and the food stuff. Arsenic, lead, mercury, cadmium and zinc can cause food poisoning. In addition copper (Cu), nickel (Ni), iron (Fe) and chromium (Cr), even in very small amounts, can act as catalysts in oxidation reactions. All of these metals may be present in stainless steel. The interaction between metal and food is also affected by the duration of contact between the food stuff and the equipment (Whitman, 1978). Brass and bronze plumbing piping can result in copper, lead and zinc contamination of water (Schock and Neff, 1988). Contamination of food can also occur in catering operations using ceramic and enameled utensils, decoration and printing used on food containers,

beverage containers, plastic containers and cardboard containers, inks and dyes used on packaging materials and wrapping papers, food colors and food flavors.

Table 1.2.1. Maximum tolerable limits for trace elements (mg kg^{-1} or mg L^{-1}) in cattle feed, milk, soil and plants (Adapted from; Bowen, 1979; Underwood and Suttle, 1999; AOAC, 1989; NRC, 2005; Hooda, 2010).

Elements	Soil	Plant	Cattle feed	Cattle milk	Water
As		---			0.01
Cd	0.06			0.006	0.001
Co	8	1	0.1	0.018	Nd
Cr	100			0.009	0.002
Cu	30	5	50	0.01	1-3
Mo		2	6		
Mn	850	0.6	40		
Ni	50		5	0.01	0.02
Pb	10		30	0.025	0.01
Se		500	0.1		0.01
Zn	50	5	40		<3

Deficiency and toxicity of some heavy metals in dairy cattle

Cadmium

Cadmium is non essential for plants, animals and humans (Sharma et al., 1979). Nevertheless, Cd is environmentally toxic element when present at concentration greater than maximum tolerable limits for Cd in cattle's diet. Cadmium toxicity may cause softness of bones, irreversible renal damages and other severe nutritional and physiological problems in cattle (Rahimi and Rokni, 2008). Cadmium toxicity in dairy cattle is rarely observed because Cd is usually present in low content in their diet. Nevertheless, irrespective of the concentration of Cd found in diet, Cd accumulation is observed in kidney and liver than muscle and milk of grazing and farmed cattle (Flanjak

and Lee, 1979). Cadmium toxicity is reported in cattle liver when ingesting Cd contaminated soil or grazing in pastures receiving successive application of phosphate fertilizer and sewage sludge or near Pb and Zn mining sites (Underwood, 1977). Cadmium content in the lithosphere is 0.06 mg kg^{-1} (Bowen, 1979). However, total Cd content in Peshawar soils is found to be varying between 0 to 3.3 mg kg^{-1} (Siddiqui & Khattak, 2010). Soil which have Cd greater than 0.06 mg kg^{-1} are considered to be intolerable for grazing cattle (IPCS, 1992; NRC, 2005). When cattle grazing in soil near Cd and Zn smelter which contains more than 1 mg of Cd per kg, a significant portion of Cd accumulates in liver (Hooda, 2010). Soils receiving high doses of phosphate fertilizer and sewage sludge usually have elevated levels of Cd (Siddiqui and Khattak, 2010). Cattle grazing in pastures near smelter are found to be more vulnerable to Cd contamination than cattle grazing in uncontaminated pastures (Koh and Judson, 1986). Heffron et al (1980) reported that greater accumulation of Cd was found in the liver, kidney and muscle of sheep fed on corn silage grown in Cd contaminated soils than non contaminated soils. In another study Sharma et al (1982) studied Cd content in muscle, kidney, liver and milk of cattle fed on Cd contaminated feed and concluded that Cd content remained within the maximum allowable limits for Cd in milk and muscle whereas greater content of Cd was found in liver and kidney. They concluded that accumulation of Cd usually occurs in liver and kidney and transfer of Cd from diet to milk was not observed in this study. In contrast to that cattle are more vulnerable to Cd contamination through air borne pollution. Clinical symptoms in cattle appear through inhalation of air (particulate matter) with <0.01 to 0.35 µg of Cd m^{-3} (Sharma et al., 1979; Bowen, 1979). The effect of Cd contaminated diet on milk production has not been

reported previously. Nevertheless there are evidences in the previous literature that Cd contamination decreased milk production (Suttle Field, 1970). Miller et al (1967) reported that supplementation of 3 mg of Cd as $CdCl_2$ in capsule may result in the reduction of milk production. Korkeala et al (1984) reported that supplementation of 0.007 mg of Cd per kg decreased the level of lactic acid in milk. Smith et al (1991) reported that increase in milk production from diet has not been observed in cow.

Infants are more vulnerable to Cd contamination through drinking Cd contaminated milk due small body and high Cd dose. .

Cobalt

Cobalt plays an essential role in the synthesis of cobalamin or vitamin B_{12} in ruminants (Winter et al., 1977). Vitamin B_{12} contributes significantly in the formation of folate in liver and also maintains the production of methylmalonic acid and level of homocysteine in plasma (Stangle et al., 2000). Lack of Co in cattle diet restrict vitamin B_{12} production in their body hence symptoms of depressed appetite, loss in body weight and listlessness arise (Josland, 1937). Cobalt deficiency in cattle is overcome through oral intake of super nutritional diet. However, there are contradictory evidences in the previous literature regarding the levels of daily intake of super nutritional Co diet to cattle. Some researchers reported that the super nutritional diet with 0.17 mg of Co per kg to dairy cattle cannot increase the synthesis of vitamin B_{12} in cattle whereas others reported that around 0.12 mg kg^{-1} of Co will be sufficient to produce vitamin B_{12} (Selinus, 2005; Tiffany and Spares, 2005; Kincad and Schoa, 2007). The difference in the response of Co in the above mentioned studies can be explained by the fact that in some cases most of the Co is incorporated in the liver rather than to be utilized for the synthesis of vitamin B_{12} .

Cobalt content in dairy cattle feeds varies from 0.11 to 0.18 mg kg^{-1} with the variety of feeds. Low energy feed e.g. wheat or rice straw and grasses required 0.11 mg of Co per kg, whereas high energy feed such as corn based silage have a requirement of 0.16 to 0.18 mg of Co per kg (CISRO, 2007). In addition to that Co requirement of grazing cattle depends on its content in the soil. For example sandy soils, poorly drained soils or soils with low organic matter are usually deficient in Co (Siddiqui and Khattak, 2010). Therefore grazing cattle are found to be low in Co because of ingesting soils deficient in Co (Underwood and Harvey, 1937). Nonetheless grazing cattle ingesting 10 g of soil do not develop Co deficiency (McDowell, 1987) Cobalt content in soils varies from 0.25 to 1 mg kg^{-1}. Farm soils or pastures soils with less than 0.25 mg of Co per kg are found to be deficient in Co (Webster and Rivoirard, 1991). Cattle grazing in such soils are usually low in Co (Milchunas et al., 1998). Like soils, pastures are found to be low in Co than legumes. Pasture requirement of Co is 0.08 mg per kg whereas less than 0.04 mg of Co per kg in the pastures will reduce vitamin B_{12} production in the cattle (McDowell, 1996). Cobalt causes depressed appetite, loss in body weight and listlessness if the daily cattle diet contains Co in levels higher than 0.86 mg kg^{-1} (Josland, 1937; Endrews and Hart, 1962; Marston, 1970; McDowell, 1997). Cobalt toxicity is very rare as Co is present at low concentrations in cattle diet, however Co toxicity can be evident in grazing cattle ingesting Co contaminated soils, or drinking Co contaminated water or eating forages grown on Co contaminated soils. However, all livestock can tolerate Co at concentrations more than 10 mg kg^{-1} in their diet (NRC, 1990). In addition to that Becker and Smith (1951) reported that no toxicity symptoms of Co were apparent in sheep consuming 150 to 170 mg of Co per kg in their diet. Unlike sheep, cattle are found to be more susceptible

to toxicity of Co when present at toxic level in their diet (Howell, 1996). Cattle showed anemia when high Co was added in their diet than its normal requirement (NRC, 2005). Cattle supplemented with diet deficient in Co usually produce milk low in vitamin B_{12} (Judson et al., 1997; Underwood and Suttle, 1999). Frobish and Davis (1977) performed a study in which dairy cattle were fed on high grain feed low in Co. They found that cattle developed low milk fat syndrome. This is most probably because low quantity of Co in the cattle diet causes reduction in the production of vitamin B_{12}. Tiffany and Spares (2005) reported that the synthesis of vitamin B_{12} reduced, in cattle fed with feed low in Co than corn silage. Kincad and Schoa (2007) carried out a study on the requirement of Co in dairy cattle fed on super nutritional and grain feeds. They found that the requirement of Co is 0.17 mg kg^{-1} in dairy cattle supplemented with super nutritional diet (supplemented with vitamin B_{12}) and it does not increase the milk production whereas around 0.12 mg of Co per kg in grain feed taken up by cattle will be sufficient for high milk yield. They concluded that type of feed is more important for milk production than concentrations of Co in the diet.

Infant requirement for vitamin B_{12} is not more than 0.3 µg per day (Collins et al., 1951). Cobalt deficiency is reduced when 0.02 to 0.05 µg per day of Co is taken daily. Infants drinking Co deficient dairy milk are considered to be more susceptible to Co deficiency than adults. As the main source of vitamin B_{12} is Co therefore its deficiency in infants causes parental megaloblastic anemia rather than drinking Co deficient milk.

Chromium

Chromium occurs naturally in trivalent form, whereas hexavelant form of Cr was introduced through industrial processes (NRC, 2005). The most commonly occurring

form of Cr is trivalent which is an essential constituent of organometallic molecule known as glucose tolerant factor and plays an important role in regulating the activity of insulin in cattle (Schwarz and Mertz, 1959; Mertz, 1993; Burton, 1995; Burton et al., 1996). Chromium Cr deficiency in animals may cause impaired glucose tolerance although the level of insulin remained normal within their body (Levine et al., 1968). The other symptoms include loss of weight and growth particularly in calves (Toepfer et al., 1977; Govinderajo et al., 1989).

The adequate level of Cr in diet remained an area of interest for the scientists as some reported that less than 1.6 mg of Cr per kg of diet increases the milk production whereas others found that 0.5 to 10 mg of Cr per kg added as a supplement in forages enhance the immunity in cattle (Besong et al., 1996; National Research Council, 1995). Unluckily till now the researchers are unable to establish an adequate level of Cr in diet for cattle essential for normal growth and milk production. Nevertheless, it has been reported that maximum tolerable levels of Cr in livestock diet ranged from 1000 to 3000 mg kg^{-1} (Yang et al., 1996).

Cr enters into diet mainly from soil where fodder is grown. Soils contain Cr less than 100 mg kg^{-1} are considered to be normal whereas soils derived from serpentine rocks or soils near industrial sites are found to be enriched in Cr (Siddiqui, 2011). Grazing livestock grazed in such soils contains elevated levels of Cr in their body. Similarly, pastures near industrial sites usually show elevated levels of Cr in their body. In addition to livestock fed on a diet grown in soils contaminated with wastewater contains high level of Cr in milk (Dey et al 1996). Somasundaram et al (2005) found out that Cr content in forages grown in soils sporadically contaminated with wastewater was 134.8 mg kg^{-1}. Chromium

toxicity is commonly observed in cattle when present in hexavalent form. Chromium deficiency may increases the glucose content and ketosis hence milk production reduces (Yang et al., 1996). Puls (1994) reported that Cr deficiency in lactating cow causes increased incidence of ketosis and decreased in milk production. There are contradictory evidences in the previous literature regarding the adequate level of Cr for cattle. Burton et al. (1993; 1994) reported that when Cr was given to newly weaned stressed calves as a supplement at 0.5 mg Cr kg^{-1} of diet for 30 days, it accelerated the response to antibody titer and enhanced the primary and secondary response to antibodies in dairy cow. Contrary to that Kegley and Spears (1995) found no response in cow supplemented with 0.4 mg of Cr kg^{-1} of diet. The difference in the immune response of dairy cows supplemented with Cr may be because Cr is present in cow body. Nevertheless, this inconsistent response to Cr supplementation in cows is yet to be explained.

Copper

Copper is essential for several enzyme system in cattle body (Harris, 1997). Copper deficiency in cattle diet causes loss of hair pigmentation particularly around the eyes (Underwood, 1991). Maximum tolerable limit for Cu in cattle diet is 40 mg kg^{-1}. Bradley (1993) and Auza (1999) observed Cu accumulation in cattle liver when fed on diet contains 4 to 5 folds greater content of Cu than maximum tolerable limit. Blanco-Penedo et al (2009) reported that Cu toxicity was observed in cattle liver when Cu content increased 2 to 10 folds in cattle diet. Grazing cattle or farmed cattle fed on forages grown in contaminated soils usually accumulated elevated levels of Cu in their liver (Miller, 1979). Sidhu et al (1994) reported 36.8 mg of Pb kg^{-1} of fodder grown in contaminated soils near industrial area. Dobraznski et al (1994) reported that grazing cattle show

elevated levels of Cu in liver because of grazing in Cu contaminated pastures. Reisbeck et al (2006) reported elevated levels of Cu in cattle fed on green forage with high level of Cu. The effect of eating Cu contaminated diet on cattle milk production has been reported previously. Perrin et al (1990) reported that milk production was reduced two fold in dairy cattle after eating Cu contaminated feed. Murphy et al (1977) reported that increased level of Cu in cow milk is from eating Cu contaminated diet. Hackbart et al (2010) reported increased milk production when cows were fed with greater content of Cu in their diet.

Lead

Lead is non essential micronutrient for cattle. Nevertheless Pb contamination is more commonly observed in farmed cattle than grazing cattle (Blakley, 1984; Suttle, 2010). Lead accumulates in the reticulum of cattle and hinders the functions of rumen microbes (Underwood, 1977). The main source of Pb contamination in farm cattle is paint and batteries. Neathery and Miller (1975) reported Pb poisoning among cattle because of eating green forages grown in soil used for dumping of old batteries. In addition to that ingesting Pb contaminated soils may cause Pb poisoning among cattle (Moffat, 1993). Lead accumulates in plant roots and transformation of Pb from roots to shoots is rare, therefore Pb poisoning through pastures among grazing cattle is rarely noticed. Nevertheless, pastures near Pb mining sites contain Pb concentration in hundreds than <0.3 mg per kg in the pastures near or in uncontaminated sites (Longhurst et al., 2004; Rajaganapathy, 2009). Burrow (1981) reported cattle death because of grazing in pastures near Pb mining sites of UK. Lead poisoning is noted among grazed and farmed cattle grazing or feeding on a diet grown on soil receiving continuous application of sewage

sludge (Saad and Fahmy, 1996). Lead toxicity among farmed cattle near highways is commonly observed than farmed cattle far away from main road. Parkpian et al (2003) reported that Pb and Cd poisoning was observed in grazing cattle near highways than away from highways. They reported that this is because of ingesting Pb contaminated soils.

Forage induced Pb poisoning in cattle milk has not been reported in the previous literature because cattle accumulate Pb in their bones rather than in their milk (Harding, 1995). Lead contamination in milk because of eating or grazing over Pb contaminated pastures has not been reported previously. Nevertheless there are evidences in the previous literature that Pb accumulation was observed in the liver and muscles of cattle grazing or fed on Pb contaminated diet. However, impact of eating Pb contaminated diet on milk quality and production is yet to be explained.

Nickel

Nickel is essential for cattle because it controls the metabolism of rumen and acts as a co-enzyme (Spears, 1984). Maximum tolerable limit for Ni in cattle diet is 50 mg kg^{-1} (NRC, 1981). Oscar and Spears (1988) reported that Ni content up to 50 mg kg^{-1} of cattle diet shows no adverse effect in cattle. Nickel toxicity in cattle has not been reported previously but air borne Ni contamination can cause respiratory tract infection (NRC, 1975; Nielsen, 1987). Nickel toxicity through diet is yet to be explained as O'Dell et al (1971) reported that daily intake of Ni content of 145, 365 and 1835 mg kg^{-1} caused no adverse effect on dairy cattle health. Forage induced Ni toxicity on cattle health needs further investigation.

Table 1.2.2. Deficiencies and toxicity symptoms in forage plants and animals because of trace elements (Adapated from NRC, 2001; Barnes et al., 2002).

Trace elements	Deficiencies and toxicity symptoms appear in forage plants	Deficiencies and toxicity symptoms appear in animals
Essential for plants and animals		
Copper (Cu)	Not reported	Loss of hair, pigmentation, scours, anemia, reduced immune functions
Nickel (Ni)	Not reported	Reduced growth, low rumen urease activity in lambs
Non essential for plants but essential for animals		
Cobalt (Co)	Impaired legume nodulation	Vitamin B_{12} <0.3 μg L^{-1} chronic wasting disease, unthriftiness and loss of weight
Chromium (Cr)	None	Not observed
Non essential for plants and animals but are toxic		
Cadmium (Cd)		Deficiency not reported but toxicity causes softness of bones, irreversible renal damages and other severe nutritional and physiological problems
Lead (Pb)		hinders the functions of rumen microbes, in severe cases causes death

1.3. Sources of Pesticides contamination in milk

Pesticides are artificially produced chemical compounds used to control organisms which may adversely affect public health or organisms which attack food and other materials essential to mankind. Pesticides is a general name given to all of them and this group includes insecticides, rodenticides, nematicides (common ones lethal to humans and plants are 1,3 dichloropropene, 1,2 dibromoethane), fungicides (e.g organomercurial compounds and hexachloro benzene for seed dressing, dithiocarbamates for vegetables and ornamental plants, pentachloro nitrobenzene used on soil before plantation), weedicides and herbicides (Diuron, paraquat, 2,4 dinitroaniline derivatives, chlorophenoxy acid derivatives and some contain dioxin impurities which are known

teratogenic). Most commonly used in agriculture are insecticides and as such they are the ones which pose a hazard to human health.

Pesticides consumption has been increased dramatically since green revolution all over the world. Pesticides are commonly sprayed over pastures and rangeland, crops, storage grains to control pest, weeds and insect attack, whereas on livestock and in the dairy farms to control antibacterial, parasitic effect, to kill rodents and rats. Pesticides are beneficial for crops but their successive application and improper doses produce deleterious effect which may result in pesticide poisoning in cattle. Significant portion of pesticide remains in soil and enter into livestock food chain by ingesting contaminated soil or eating contaminated diet (Carson, 1962). Pesticide residues are reported in the dairy milk and milk products of cattle eating pesticide contaminated diet or over a long period of time (Ivie and Dorough, 1977; Waliszewski et al., 1997; Tariq et al., 2007). Cattle when exposed to pesticide contamination usually develop clinical symptoms (Table 1.3.1) and in some cases acute pesticide poisoning results in death (NRC, 1975; Rudd and Genelly, 1956).

Due to successive application and inappropriate doses of pesticides, their residues remain in soil and grasses hence grazing cattle when ingesting 1 kg of soil per day alongwith fodder are directly exposed to pesticide residue from soil than farmed cattle (Friberg et al., 1992). Pesticides have a very long half-life in the environment and therefore they can produce hazardous effects on humans and livestock. Organo chlorine pesticides are considered to be toxic and are prohibited in developed and developing countries. Nevertheless they are commonly used in under developed countries to control pest and weed attack on crops, pastures and rangeland.

Table 1.3.1 The LD_{50} of pesticides and their lethal effect on external parts of livestock (adapted from Code of Federal Regulations, 2010).

Pesticides vulnerability	Toxic levels of pesticides (mg kg^{-1} or mg L^{-1}) taken up by livestock			
	High	Moderate	Slight	Almost non toxic
Oral LD_{50}	50	50-500	500-5000	>5000
Inhalation LD_{50}	0.2	0.2-2	2-20	>20
Dermal LD_{50}	200	200-2000	2000-20000	>20000
Effect on Eyes	Irreversible corneal opacity for at least 7 days	Reversible corneal opacity within 7 days, irritation for 7 days	Only corneal irritation for upto 7 days but no opacity	No irritation
Skin	corrosive	Sever irritation lasting for 72 hrs	Moderate irritation lasting for 72 hrs	Mild or slight irritation lasting for 72 hrs

Pesticides have made great improvements in agriculture and human health. Harm occurs due to their misuse or ignorance albeit some environmental damage is inevitable. Indiscriminate and rapid use leads to development of resistant population. In developing countries pesticides are a very common cause of contamination of potable water second only to organic sources (Lagaly, 2001). Currently 1000 pesticides are in use world over. Most countries have adopted strict regulations regarding usage and have therefore adopted Maximum Residues Limits (Duffus, 1983). MRLs are well below perceived safety level and require sensitive analytical techniques (Elzerman and Paddock, 2000). About 50 organo phosphates are currently approved for use in United States by US EPA.

Insecticides are divided into the following groups:

Chlorinated hydrocarbons

They are the most widely used and thus environmentally most prevalent. The most common of these are DDT, & its metabolites DDD, DDE, lindane, heptachlor, aldrin and dieldrin. DDT, lindane and hepatochlor have half lives of 10+ years while that of DDE is in decades. Aldrin and dieldrin are less persistent and they remain in the soil for 2 ½ years before 95% degradation. DDT, aldrin and dieldrin have been banned in USA and UK but still used in tropical countries because of their low cost. The adverse effects of DDT are due to its extensive use where it finds its way to water as surface runoff. However, DDT has the ability of bio-accumulation. This is evident from bioaccumulation of DDT in food chain from 0.001 ppm in sea water rising upto 700 ppm in oysters and even more in higher animals. DDT has affinity for adipose tissue where the turnover rate is low unless in conditions of stress. The accumulated DDT causes harmful effects in blood. The harmful effects of DDT were first noted in birds of prey where impaired calcium metabolism lead to thin eggs shell and as such reproductive failure. Also behavioral changes occurred which favored egg breakage by parents. The same problem occurred in boiler chicken where sawdust treated with DDT was used as litter. Harmful effects were also noted in fish including behavioral changes leading the reproductive difficulties, increased mortality among young ones and acute toxicity to adults. Small concentration of about 0.01 ppm reduces photosynthesis in marine plankton while even smaller concentration of 0.001 ppm in sea water kills shrimps. Poly chlorinated biphenyls (PCBs) are similar to DDT but more resistant to acids and alkalis, stable up to 500^0C and sparingly soluble in water and therefore they are used extensively. They may leak into the

environment due to poor waste disposal techniques. They are also persistent in the environment and fat soluble and therefore produce effects similar to DDT like carcinogenicity and mutagenecity (Duffus, 1983).

Excessive use, persistence in the environment and carriage upto distant areas leads to development of resistance and effect on non-target organisms.

Organophosphate compounds

These are derivatives of phosphoric acid and include malathion, parathion, diazinon, dichlorvos, chlorpyrifos, profenofos, etc. They are rapidly metabolized by mammalian enzymes and rapidly degraded in the environment and as such cause a localized damage. Since they are non-persistent, they don't bioaccumulate. Their mechanism of action is by inhibiting acetylcholine esterase, as a result of which the action of acetylcholine at the new endings continues infinitely with each nerve impulse. This results in tremors, convulsions and death. Certain organophosphates such as sarin, tabun, etc are used as a nerve gas chemical warfae agents. Howver, low mammalin toxicity organophosphates are used in a number of pest control operations as a replacement of chlorinated hydrocarbons. However resistance is also developing against them (Maxcy and Rosenau, 1984).

Carbamates

These are derivates of carbamic acid and act by inhibiting acetyl cholinesterase. They also have short half life, low persistence in the environment, rapid degradation and least effect on non-target organisms. But they have a quick knock down effect. Common ones include carbaryl, propoxyur (Maxcy and Rosenau, 1980).

Pyrethroids

Pyrethroids are synthetic pyrethrins. Natural pyrethrin are the insecticidal constituent of pyrethrum flower (chrysanthemum). Chrysanthemum flower contains 5 different types of compounds with insecticidal properties; these are pyrethrin I and II, Cinerin I and II and jasmolin II. They are unstable to sunlight, air, moisture, alkalis and rapidly degraded after application. They undergo very rapid metabolism and as such pose low mammalian toxicity. However they can cause local and systemic allergic reactions, nausea, vomiting, headache and CNS disturbances. Synthetic pyrethroids like cypermethrin are photostable and are effective in agricultural and household pests control. The pyrethroids do not show environmental persistence and show very rapid insect knockdown. (Duffus, 1983).

Fumigants

Fumigants are inhaled by respiratory tract and then kill the insect. Commonly used include methyl bromide and phosphine (PH_3). They are highly toxic and should be used in closed areas. Methyl bromide is lethal in 50 mg / body wt. dose (Ilyas, 2003).

1.4. Justification

Heavy metals and pesticides gain entry into dairy milk through the diet of the cattle. The feed of the cattle in our country consists of cotton khal, maize khal, sugarcane plants, maize plant and fruit, wheat bran, alfaalfa grass. Amongst them cotton, maize and sugarcane are especially sprayed with pesticides because of the abundant pests in them. Heavy metals can also gain entry into the body of cattle through drinking water as in most of the areas cattle owners do not have scientific farms and they take the cattle out to drainage channels or canals where the possibility of mixing of industrial waste water containing toxic heavy metals cannot be ruled out. Since heavy metals and pesticides are

known to produce long term toxic effects on human body therefore their presence in milk even in small amounts may be detrimental in the long run. This is more due to the fact that these substances are stored in adipose tissue of body and show bioaccumulation and biomagnification. Therefore it was felt imperative to ascertain if toxic heavy metals and pesticides are present in dairy milk in the study area. Couple of studies have been done on heavy metals in milk in Pakistan and a few on heavy metals and pesticides in vegetables and fruits but no such study has been conducted on dairy milk which is a very commonly consumed item in our country. As such this study has been designed to assess common toxic heavy msetals like copper, cadmium, chromium, lead, nickel and cobalt and pesticides like cypermethrin, chloropyriphos, and prophenofos in dairy milk.

1.5. Objectives

- To assess the levels of toxic heavy metals (Cd, Cr, Co, Cu, Ni & Pb) in dairy milk of Peshawar district and to compare them with maximum residue limits (MRLs) of these elements as given by WHO/FAO for any possible harmful effects on humans.
- To assess the levels of GC-amenable pesticides uptake by plants used as animal feed (cypermethrin, chlorpyriphos, & profenophos) in comparison to the dairy milk of Peshawar district and maximum residue limits (MRLs) of these compounds determined by WHO/FAO for any possible harmful effects on humans.
- To assess the levels of same toxicants in animal feed and correlate it to levels in milk.
- To suggest any remedial measures if possible

Chapter 2

LITERATURE REVIEW

2.0. REVIEW OF LITERATURE

2.1 Dairy Farming In Peshawar

Dairy farming is a very important industry in Pakistan because Pakistan's stands 5th in the world with respect to milk production. GDP of dairy sector has been increased from 9% to 11.3% over a period of last decade (Economic Survey of Pakistan, 1999-2000 & 2010-11).

Dairy farming system in Peshawar is a combination of mixed farming, urban and peri-urban dairy farming. Mixed farming is commonly used in rural areas where small numbers of cattle (up to 10 in numbers) are kept in herds. Around 57% of cattle are kept in this system (Sarwar et al., 2002).

2.2. Heavy metals

Heavy metals or trace elements are present naturally in low concentration (Bowen, 1979). Some of the metals like Cu, Cr, and Zn are considered to be essential for plant growth and also required for normal body development. Contrary to that some of these elements for example Cd, Hg and Pb are considered to be non essential for plant growth and body development but are toxic when present at concentrations greater than their normal value in plant or human body (Alloway, 1995). Heavy metals cause neurotoxicity, nephrotocicity, fetototxicity and teratogenic toxicity in humans when exposed via eating contaminated food, drinking contaminated water, inhalation of contaminated dust, ingesting contaminated soil or via skin contact. Once they enter into human, body these may causes disturbance in the blood and cardiovascular system, changes in the detoxification pathways (colon, kidney, liver, skin), release and functioning of various

hormone, disturbances in gastrointestinal, reproductive and nervous systems. As a consequence of that, changes in the behaviour of mental and neurological functions, alteration in neurotransmitter production and utilization are commonly observed among humans directly exposed to heavy metals contamination (Hu et al., 2002). Heavy metals cause imbalance in Ca content in blood resulting in osteoporosis, inflammation in arteries and tissues. Apart from heavy metal toxicity in human body, scientists are more concerned about increase in the free radicle activity in our body when the concentration of heavy metals exceed than their normal requirement for our body. Free radicles are negatively charged, highly reactive and unstable molecules with free unpaired electrons. Free radicles play an important part in various metabolic activities (Fang et al., 2002).

Toxic metals are removed from human body through Chelation. Chelation therapy involves the removal of attached metals from the body tissues which then enter into the blood and from their, they reached to the kidney and finally are excreted through the body via urine (Pouls, 2009).

2.2.1. Heavy metals in soil, water and fodder

Heavy metals are commonly found in small concentrations in soil. This is because natural processes of wear and tear are constantly acting on the rocks and this also leads to the formation of soil. The artificial sources of heavy metals in soil are mining of minerals, effluents from industry, disposal of domestic, municipal and hazardous waste (Alloway, 2005). Some metals are essential in minute quantities for all living organisms and many soils do not contain them in sufficient quantities. They are considered to be toxic to plants and soils when present in greater concentrations than their normal values in soils. When

fodders are grown over contaminated soils it becomes a source of metal toxicity in cattle (Bowen, 1979). Imadullah et al (2001) found out that the mean levels of Cd, Cr, Cu, Ni and Pb in soil were 0.06, 2.06, 7.0, 5.71, 1.19 mg kg^{-1} in of Peshawar and Nowshera districts of Pakistan. According to Nasreen (2006) the average concentrations of Cd, Cr, Cu, Ni, Pb and Zn were 1, 40, 3, 3, 10 mg L^{-1} in tube well water samples of Peshawar district of Pakistan. Siddiqui and Khattak (2010) gave the mean content of Cd, Cr, Cu, Ni, Pb and Zn as 1.7, 80, 25, 40, 18 and 55 mg kg^{-1} in the soils of Peshawar district of Pakistan. Jan et al (2011) found that the levels of Cr, Cu, Ni and Pb were 0.025, 0.022, 0.015 and 0.082 mg kg^{-1} in the fodder grass of Peshawar district of Pakistan.

2.2.2. Heavy metals in food chain of human and cattle

Food chain transfer of heavy metals takes place near smelting or mining areas, agricultural land, pastures grown over contaminated soils. Cai et al (2009) studied the Cd and Zn contamination in cattle grazed over the pastures near Cu and Zn smelter. They found that the concentration of Cd, Cu and Zn was greater than their normal content in cattle milk and muscle. They concluded that this is more likely because of drinking and eating contaminated water and food. As a result of heavy metal contamination of soil there is a direct entry in the food chain of living organisms through several routes such as food, inhalation or ingestion.

2.2.3. Heavy metals in milk

Licata et al (2004) studied the concentrations of trace and toxic elements in dairy milk of Italy. They found that the content of As, Cd, Cr, Cu and Pb was 37.90, 0.02, 2.03, 1.98

and 1.32 µg kg^{-1}. They concluded that greater content of As and Pb in milk is more likely because of drinking contaminated water. Awan et al (2005) carried out a study in Pakistan. They reported that the concentration of Pb varied between 0.04 & 0.019 mg kg^{-1} and between 0.081 to 0.143 mg kg^{-1} in raw and pasteurized milk samples of dairy cattle. They found that Pb content remained greater than WHO maximum residue limits of Pb (0.025 mg kg^{-1}). They concluded that greater content of Pb in raw and pasteurized milk samples is more likely because of eating Pb contaminated diet.

Dobranznski et al (2005) carried out a study in Poland where he analyzed dairy cow milk samples for trace elements. They found that Ba, Cu, Cr, Co were 191.21 – 224.85, 89.85 ± 125.14 - 65.37 ± 85.3, 88.13 ± 59.33 - 75.06 ± 44.80 , 8.34 ± 3.55 - 5.16 ± 2.79 µg L^{-1}. Hosnedlova et al (2005) studied the content of Cu and Zn in dairy cattle milk of Bohemian Region of Czech Republic. They reported that Cu content ranged from 5, 5-50 and 50 µg L^{-1} in 57, 14 and 28% of milk samples. Copper content in dairy milk samples remained within the maximum residue limits as reported by EU (2006). They concluded that milk was deficient in Cu and this is more likely because of low content of Cu in diet given to dairy cattle. Contrary to that Anastasio et al (2006) carried out a study in Italy and found that Cd and Pb contents in dairy milk were greater whereas Cr content remained less than their WHO maximum residue limits.

Florea et al (2006) carried out a study in Romania. They found that the concentrations of As, Cd, Cr and Pb in dairy cattle milk samples of Romania remained less than maximum residue limits of 0.05 mg kg^{-1} for Cd and Cr and 0.02 mg kg^{-1} for Pb. Abdallah (2009) studied trace element content in dairy milk of Egyptian Cattle. They reported that Cd, Hg

and Pb content in dairy milk samples was greater than their maximum residue limits as suggested by Egyptian Govt (see Table 2.1).

Table 2.1. Metal content in dairy smilk samples reported by Abdullah (2009).

Elements	Dairy cattle milk samples (mg kg^{-1})	Maximum residue limits (mg kg^{-1})
Cd	0.41	0.05
Cu	0.11	0.4
Hg	0.10	0.02
Pb	2.55	0.3
Zn	3.66	5.0

2.2.4. Toxicological consequences of heavy metals

2.2.4.1 Cadmium

Sources

Cadmium occurs naturally in phosphate rock whereas sedimentary Ca phosphate rocks and marine black shales usually contain highest content of Cd ranged from 15 to 300 mg kg^{-1} (Hooda, 2010). The other sources or anthropogenic sources of Cd in soils are sewage sludge, phosphate fertilizer obtained from sulphate ores and mining and smelter of Pb and Zn (Alloway, 1995).

Uses

Cadmium is used commercially for antirust coating, electro plating, protective coating of engines of vehicles, steel stabilizers, stabilizer in plastics, pigments in paints, glasses and plastics, cadmium copper alloys in high conductivity cable wires, in bearing alloys and auto mobile components and also as an electrode component in nickel cadmium batteries. Cadmium forms compound with inorganic salts such as chloride, sulphate and acetate and complexes with organic compounds (Friberg et al., 1992).

Cadmium in food

Cadmium concentrations differ in different food stuff. Cadmium content is present at high level in meat offal, sea foods, animal liver and kidney naturally and may cause toxicity when present at high concentrations. Once it enters into the food chain of human beings and animals, it causes severe health hazards (Fitzgerald, 1985; Vreman et al. 1986). Livestock are exposed to Cd contamination when fed on diet grown over soil contaminated with sewage sludge or industrial effluents or received heavy doses of phosphate fertilizers (Hammer et al. 1971). Cadmium is non essential for plant and animals but is toxic when present at high concentration (Vos, 1987).

High content of Cd in environment causes no adverse effect on human health. Nevertheless, Cd causes dysfunction of kidney when its concentration increases to 200 mg kg^{-1} body weight in human kidney. However, acute toxicity was observed in Japan a century ago in an incident where daily intake of Cd was more than 75 mg kg^{-1}. Finally it was discovered that the main cause of human death was due to eating Cd contaminated rice. Blunden and Wallance, (2003) reported death of many adults mainly due to eating

Cd contaminated food from contaminated Zn coated tins. Asami (1984) found that addition of municipal sewage sludge in soils used for growing rice was the main cause of Cd accumulation in the human kidney. ATSDR (1989) reported that the tolerable daily intake of 0.075 µg of Cd per day per kg of body weight may cause no adverse effect on human and animal health. However, Cd toxicity is usually found when dietary tolerable Cd limits increased from 0.075 µg per day per kg of body weight of human (Gross et al., 1976). Ostergaard (1977) reported that the concentrations of smoking can add extra 20 to 35 µg per day in their intake.

Absorption of Cadmium in human body

Cadmium when enters into the body through diet accumulates in fat tissues and proteins and persists for long durations. Hammer et al. (1971) reported that Cd may accumulate in body tissue for more than 40 years without being removed from the body.

Effects of Cadmium on Human body

Cadmium causes severe adverse effects on human health. Nausea, vomiting, headache, abdominal cramps and liver damage, renal damage, bone damage, effects on testis, immune system and cardio vascular system and cancers is observed in human when exposed to Cd contamination (Browning, 1969).

Carcinogenic potential

According to U.S. Environmental Protection Agency (1989) report cadmium inhalation may cause lung cancer among the workers exposed to Zn and Cd mining. Nevertheless cacinogenic effect of Cd was not observed among them. Cadmium III can bind to nucleic

acids and causes abnormal synthesis of genetic material in cells and hence is mutagenic. Cadmium is considered to be a potential carcinogen because of inhalation. Therefore Cd content should be decreased in ceramic and glass industries (US EPA 1989).

2.2.4.2. Cobalt

Sources

Natural source of Co is volcanoes, eroded materials and burning of forests whereas anthropogenic sources are phosphate fertilizer, smelting and mining, refining and processing of Co alloys, and industries (Hooda, 2010).

Uses

Cobalt is used for preparation of high strength alloys, permanent magnets, manufacture of glass and glazing for pottery. It is a rare element and makes up only about 0.001% of lithosphere (Bowen, 1979).

Cobalt in food

Cobalt enters into food chain of humans through milk and dairy products, fish and crustaceans and condiments, sugar and oils. Cobalt content in green leafy vegetables ranged from 20 to 60 mg per 100 g of food whereas organ meat and muscle meat having Cd varies from 15 to 25 and 7 to 12 mg per 100 g of food (Onianwa et al., 1987; Leblance et al., 2006).

Absorption of Cobalt in human body

The absorption of cobalt from food varies from 5 to 45% depending upon the body levels and presence of other minerals (Toskes, 1973). The total content of Co in human body is not more than 1 mg. Cobalt once enters in human body is excreted via urine (Yamagata 1962). Cobalt is essential for human body and is an important part of vitamin B 12, cynocobalamine. Cobalt is synthesized outside the human body by certain bacteria and fungi and cannot be synthesized within human body. Therefore it has to be taken in a preformed state. The tolerable dietary daily intake of Co is 5-40 µg per day (Reilly, 1991).

Effects of Cobalt on Human Body

Acute toxicity of cobalt: may cause asthma, pneumonia, and wheezing whereas it's deficiency leads to megaloblastic anaemia and possibly goiter (Blokhima, 1970).

Carcinogenic potential

US International Agency for Research on Cancer (1990) mentions Co as a possible carcinogen to human.

2.2.4.3. Copper

Sources

Copper is extensively found in the environment. It's sources are pollution due to Cu mining, industrial uses, solid and liquid waste of industrial and domestic origin, burning of fossil fuels, production of phosphate fertilizer, volcanic eruptions, fires and dust storms. (Public Health Statement, 2011).

Uses

Copper is used in electricity cables, copper pipes, sheets for roofing buildings, alloying with zinc, tin and cadmium, fungicides (Bordeaux mixture, Cuproarsenate). Brass (copper and zinc) is extensively used in homes and work places. Copper forms complexes with amines and other ligands (Reilly, 1991).

Copper in Food

Copper is important for all living organisms. Copper concentration in animal diet and plant is $\leq 2mg\ kg^{-1}$ (Alloway, 1995). Whole grain cereals, meat, fish and green vegetables are rich sources of Cu for human health. Refined cereals and milk products are low in Cu (Varo, 1980). Commercially prepared tinned food is considered to be deficient in Cu and cause heart disease in human (Klevay, 1975).

Absorption of copper in human body

Copper when enters into human body through food around 30% of Cu is absorbed in body tissues. Nevertheless, Cu absorption in human body can be restricted while adding phytates in their diet. However, increase in Fe, Mo, and Zn in human body accelerates Cu loss through excretas. Copper once enters into human body it usually remains in the liver, heart, brain and kidney (Boyne, 1986). Regular absorption of Cu can reduce the activity of copper homeostasis and may result into accumulation of high content of Cu in plasma in elderly (Wapnir et al, 1993).

Effect of copper on human body

Copper is essential for human body. Copper is an important part of several enzymes like cytochrome oxidase, tyrosinase, amine oxidase, uricase, superoxidase, dimutase and its deficiency may cause imbalances in the enzymatic system of human body.

Copper deficiency may cause Menke's steely hair syndrome, bone defects, hair damage, severe diarrhea and anaemia in human. Excessive amounts of copper in human body can result in copper deposits in liver and brain with resultant damage, a condition called Wilson's syndrome (Karpel and Penden, 1972).

Carcinogenic potential

In animal studies involving rats adenocarcinomas and fibromas of lungs have been detected (Stettler et al., 1988). However it has not been classified as a human carcinogen due to unavailibility of human data and inadequate animal data (Integrated Risk Information System, 1998)

2.2.4.4. Chromium

Sources

Chromium naturally occurs in serpentine rocks in high concentrations. Chromium occurrs in three forms; Cr (0), Cr (III), and Cr (VI). Chromium (III) is most commonly found in earth's crust in low concentration whereas Cr IV is found infrequently (Alloway, 1995). Chromium IV is considered to be toxic than Cr (III) (Hooda, 2010). The other sources of Cr are fly ash, chromate smelter, mine tailings. However, a significant portion of Cr is

released into air, soil or water through manufacturing and disposal of Cr containing products or chemicals. However Cr occurs in fresh vegetables and fish. Similarly cooking in stainless steel utensils may release Cr in food. High content of Cr-VI is found in drinking water become contaminated with the effluents discharge from Cr manufacturing industry (Reilly, 1991).

Uses

Chromium has been used in alloy steel over the last couple of centuries. Chromium is used as a part of stainless steel of various kinds, as an anti-corrosive in cooling systems, forms alloys of iron, nickel and cobalt, is a part of tanning agents, various color pigments, preservatives, household detergents, pottery glazes, paper and dyes (Burrows, 1983; Reilly 1991).

Chromium in food

The food which contains maximum chromium is yeast but to a lesser extent it is also found in animal meat and liver, poultry and butter, Black and Green pepper, Apples, Bananas & Spinach (Hamrick and Counts, 2008).

Absorption of Chromium in human body

Chromium enters into human body via food and around 0.5-1.0 % of Cr III and 2% of Cromites accumulate in the body tissue (Donaldson, 1966). Chromium absorption is inhibited due to the presence of Fe, Mn, V and Zn in food more likely because later elements can compete with Cr and adapt same pathway from stomach to intestine. It

accumulates in kidney, spleen, testes and bones. The main route of excretion is urine and only some in feaces. Its body content decreases with age (Hill, 1976).

Effects of Chromium on Human body

Chromium plays an important a role in body development such as in lipid metabolism, glucose tolerance and dinicotinic acid- glutathione complex (Bunker, 1984). Chromium has a role in glycolysis and ATP production. Trivalent Cr is an essential constituent of organometallic molecule known as glucose tolerant factor which is important for the functioning of insulin in cattle (Burton, 1995; Burton et al., 1996; Earley et al., 2002).

High concentration of chromium VI can negatively effect air ways, gastrointestinal tract, kidneys and can even cause death (Reilly 1991).

Carcinogenic potential

Chromium III can bind to nucleic acids and cause mutagenesis while chromium (IV) compounds are known carcinogens (Okada, 1982).

2.2.4.5. Lead

Sources

Lead in lithosphere ranges from 2-200 mg kg^{-1} . However, Pb content varies in various parts of the world (Alloway, 1995). Lead in the atmosphere comes from vehicles, industrial processes, emissions from foundries, coal and painted wood burning, emissions

from metallurgical works, erosion of lead ore bodies, emissions from smelters, volcanic eruptions, food grown on contaminated soil etc. (O'Brien., 2010).

Uses

Lead has the ability to form alloys with other metals. It is used for batteries in cars, vehicles, emergency lights (accounting to 40% of total world consumption), lead sheathed cables, lead pipes, bullets and projectiles for guns, lead solders in plumbing and electrical work, food cans, car body manufacture and bearings, lead paints, rust inhibitor for iron and steel, glazing of ceramics, glass manufacture, television tubes, fluorescent lights, drier in paint and printing ink and insecticides and manufacture of petroleum additives (Dowding, 1978).

Lead in food

Lead may enter into the food through contamination with paints, lead dust, water from lead pipes, cosmetics, food in lead coated utensils, certain candies, food cans, food colors and utensils paints (Hamrick and Counts, 2008)

Absorption of Lead in human body

Only 10% of the ingested lead is absorbed through gastrointestinal tract in adults and more than 50% in children (Kehoe, 1961). Lead absorption in body is increased by low body calcium, increased vitamin D, increase carbohydrate, iron deficiency and protein deficiency. In blood it is bound to red blood cells, 16 times, higher than in plasma (Barry, 1975).

Around 90% of Pb is removed from the body via feaces whereas 10% is excreted through urine and minute quantity is released in secretions, hair, nails and sweating (Porru and Alessio, 1996).

Effects of Lead on human body

The average Pb content varies from 10 to 35 µg 100 ml^{-1} in the blood of healthy humans and increased to 300 µg 100 ml^{-1} when exposed to Pb contamination (NRC, 1981). Lead is present in all parts of human body but usually accumulates in their bones. Healthy adult have an average content of 120 mg kg^{-1} of Pb whereas 1.4 mg 100 mL^{-1} is in blood, more than 100 mg kg^{-1} in bones while liver has 1 mg kg^{-1} of Pb in their body. Placental transfer occurs in fetus and after that lead deposit in human body increase throughout their life (Reilly 1991).

Infants and children are very quickly affected by lead than the elderly in whom the brain development processes slow down. If such foods are taken regularly which contain even small amounts of lead, it can cause harmful effects for long periods. Lead is a typical cumulative poison. Lead ions get attached to haemoglobin and plasma proteins due to which red blood cell production and resultant oxygen supply is greatly effected. Excess of lead may enter in the bone-marrow, liver and kidneys (Reilly, 1991).

Lead when enters into human body via food may lead to gastrointestinal effects such as anorexia, dyspepsia, constipation, colic and abdominal pain, encephalopathy, peripheral neuropathy, chronic irreversible nephropathy, aggressive behaviour, wrist drop, effects on liver, bones and male gonads, inhibition of haemoglobin synthesis and short life span of red blood cells (WHO, 1976).

Carcinogenic potential

IARC has placed lead in Group 2B "possible human carcinogen" and NTP has placed it as "reasonably anticipated to be a carcinogen," but Occupational safety and Health Administration does not consider it to be a "select carcinogen" (Stellman, 1998).

2.2.4.6. Nickel

Sources

Nickel is naturally present in meteorites and siderites may contain Fe alloy with 5-20% Ni. Nickel is commercially obtained from pentlandite and pyrrhotite (Alloway, 1995).

Burning of fossil fuels, emissions from mining and refining processes, steel production, electroplating, municipal waste incineration, water and soil contaminated with municipal sewage sludge, volcanic eruptions, landfill sites, vehicular emissions, seabed and earth's core (Edbon, 2001).

Uses

Nickel steel alloy is widely used in industry. Ni is also used to protect metals from corrosion and therefore is widely used in motor vehicles, armaments, aircrafts and tableware. It is also used in storage batteries, paints, pottery, cosmetics, as a catalyst in the hydrogenation of edible oils, automobile and air craft parts, electrodes, coinage, it also forms alloys with Al, Fe, Cu, Cr, Mo and Zn (Smart and Sherlock, 1987).

Nickle in food

Nickel is present in low concentration in food. Nevertheless Ni content varies from 3.9 to 8.2 mg kg^{-1} in pea nuts and legumes (more than 1 mg kg^{-1}) cacao (5-10 mg kg^{-1}), grain products and canned food (Ellen, 1987; Pennington, 1987; Smart and Sherlock, 1987). Ni is released into the air through tobacco smoke, vehicular emissions, industrial waste, fertilizers and burning. It enters into food through processing, hydrogenated oils, steel utensils, dental procedures (Park and Park, 1992).

Absorption of Nickel in human body

Nickle absorption depends on quantity taken, solubility, form (nickel carbonyl is completely absorbed). In humans 20-35% of the inhaled less soluble nickel is absorbed in the blood. Nickel is rarely absorbed from food. However, only 3 to 6% of Ni is absorbed from dietary intake in human body tissue. From the GIT absorption is affected by the composition of diet, chemical form and interactions with other elements. Nickel absorption may be suppressed by binding or chelating substances, competitive inhibitors, or redox reagents; on the other hand, absorption is often enhanced by substances that increase pH, solubility, or oxidation, or by chelating agents that are actively absorbed. It is excreted via urine. It is evenly distributed in the body (ATSDR, 1997).

Effects of Nickel on human body

The enzyme urease contains Ni. Nickel activates carboxylase trypsin and actyl coenzyme A (Sunderman, 1965). Dietary Ni is not harmful to man but inhalation of Ni may cause cancer of respiratory tract and dermatitis (Stokinger, 1963). It can also lead to skin rash,

asthma attacks, chronic bronchitis, reduced lung function, delirium, convulsions, and death (Brown and Sunderman, 1980).

Carcinogenic potential

Ni Carbonyl can cause cancer of the lungs and sinuses in industrial workers after prolonged exposure. IARC has placed Nickel carbonyl in Group 2B "possible human carcinogen", and NTP has placed it as "reasonably anticipated to be a carcinogen," while OSHA has categorized it as "select carcinogen" (ATSDR, 1997). (See Tables 2.2.4.6.6.1 & 2).

Table 2.2.4.1. Differences in the mean content of some general characteristics of heavy metals (Reilly, 1991)

Metal	Periodic no.	At. Wt	Density /sp.gr	Melting pt	Solubility	Conductivity	Oxidation state	Charactaristics
Cd	48	112.4	8.6	320.9	Organic acids, less in water	Poor	2	Malleable
Co	27	58.9	8.9	1490	Dilute acids	Poor		Hard & brittle
Cr	24	52	7.2	1860		Moderate	-2 to 6	Hard & brittle
Cu	29	63.54	8.96	1083		Very good	1,2	Tough, ductile
Ni	28	58.71	8.9	1453	Acids only	Good		Tough
Pb	82	207.19	11.4	327°C	Slight in water	Poor	0,2,4	Soft,bendable

Table 2.2.4.2. Differences in the physical characteristics of heavy metals (Reilly, 1991).

Metal	Occurrence	Soil. life	Max.limits (ATSDR, 1989; heavy metal toxicity.htm; Reilly 1991)
Cd	In combination with Zn,Cu,Pb and extracted as their by- product	Binds strongly	.4-.5 mg wk^{-1}, 0.005 mg L^{-1} in water, 100 µg per m^3 as fumes,200 $µg/m^3$ as dust, 15ppm in food colors, MCLG 5 µg L^{-1}, lethal dose 20-130 mg kg^{-1}
Co	Occurs as an ore in combination with arsenic. Also extracted as a by product of Ni,Cu,Pb	Very long	OSHA 0.1mg per m^3day^{-1} in workplace air, NIOSH 0.05 mg per m^3
Cu	Occurs as sulphides, oxides, carbonates, arsenides, and chlorides in ores along with Zn, Mb, Cd . Easily extracted from ores		30 µg kg^{-1} adults, 40 µg kg^{-1} children, 50 µg kg^{-1} water
Cr	Occurs as chromic acetate, citrate, chloride, SO_4 of Cr III and chromates & dichromates of Cr VI		100 µg day^{-1}, 50 µg L^{-1} in water, 5 µgm m^{3-1} in air
Ni	Occurs as ores with Fe,As, Sb, Sulphur, extraction is difficult	Long	occup exposure 1 mg m^3 (40 hrs wk^{-1}), OSHA- PEL limit – 0.007 mg per m^3 or 0.001ppm
Pb	Occurs as lead sulphide, sulphate and carbonate along with Zn,Fe,Ag,Cd. Easily extracted from ores	Long	430 µgm day^{-1} in adults, 3.5µg kg^{-1} day^{-1} in infants, 50 µg L^{-1} in drinking water

2.3. PESTICIDES

2.3.1. Pesticides in soil and fodder

When pesticides are applied to the soil they can undergo adsorption. Adsorption means the attachment of pesticides to soil. This depends upon the chemical nature of pesticide, certain charactaristics of soil like it's water content, pH, the amount of organic matter in it and whether it is sandy, loamy, hard or soft. Adsorption of pesticides is more in those soils which have more clay and organic matter than sandy nature soils. Livestock and crops downstream can be affected by pesticides which reach them either dissolved in water or with soil particles. These pesticides are then absorbed by the fodder producing plants and then degradation occurs. Degradation can occur even in soil and it occurs by microbial action and chemical reactions e.g hydrolysis, redox reactions or photodegradation. (Environmental Fate of Pesticides, 2010). Pesticides are commonly used to kill pest over the crops or added to soil before cultivation. However, improper use of pesticide may result in the residual accumulation of pesticides in soil and crops or grasses grown over the soil (Bruce and Richard, 1987).

Karabasanavar et al (2012) performed a study in India in which they studied the residual concentrations of chloropyrifos in animal feed and fodder samples of Tarai region of Uttarakhand, India. They found that more than 25 feed and fodder samples were contaminated with chloropyrifos. Muschal and Warne (2003) carried out a study in South Wales, Australia in which they estimated the residual concentrations of chloropyrifos, endosulfan and profenofos in aquatic organisms. They found that more than 20 aquatic organisms had residual concentrations of chloropyrifos more than the maximum residual limits.

2.3.2. Pesticides in food chain

Pesticides can reduce crop yield by reducing nitrogen fixation (Rockets, 2007). However besides nitrogen fixation , pesticides can be directly absorbed by plants with water and will cause retarded growth of the plant, with improper development of stem and roots (Walley et al., 2006). Animals can be affected by directly eating the poisoned plants or due to reduced food production (Palmer et al., 2007). Birds can be effected by eating the poisoned insects. When these pesticide laden plants, birds and animals are eaten by humans, they enter into their bodies. When human beings and other higher animals continously take pesticide laden plants and animal food for several years, these pesticides can accumulate in their body to dangerous levels and cause harmful effects. (Krieger, 2001).

2.3.3. Pesticides in milk

Pesticides enter in milk through animal food, water, air and soil. Pesticide residues have been detected in milk frequently in various researches. A study conducted by the USDA Pesticide data programme showed that 96% of the samples contained DDE, 99% DPA, 41% dieldrin, 25% synthetic pyrethroids and 9% had 3 hydroxyfurans which is a breakdown product of carbamate insecticides (Philpott, 2008).

Sassine et al (2004) studied cypermethrin contamination in dairy milk samples of Sao Palou, Brazil. Dairy cattle were sprayed with 50 g L^{-1} of active ingredient of cypermethrin and found 16 fold greater cypermethrin in cattle milk within 24 hours of cypermethrin exposure. Nonetheless, cypermethrin was undetectable after 17 days of the exposure. Another study was carried out by Battu et al (2004) in Ludhiana-India. DDT

and Lindane were 3 fold greater in cattle milk samples than their maximum residue limit (MRL), however organophosphorus or synthetic pyrethroid insecticides remained within their maximum residue limits of 0.01 mg kg^{-1} in milk samples.

Bissacot and Vassilieff (1997) studied the pyrethroid insecticides (flumethrin, deltamethrin, cypermethrin and cyhalothrin) injections given to cow in Brazil. They found that small amounts of deltamethrin, cypermethrin and cyhalothrin were present in cow's milk and blood samples immediately after injection whereas flumethrin was found after 28 days of injecting pyrethroid insecticides. According to the annual report of USDA (1995), the residues of pyrethroid and organophosphate pesticides is increased in milk samples over a period of a decade. Presence of diiphenylamine residues, dicchloro-diflouro ethane (DDE) and endosulfan in milk was reported since 2004. They concluded that this is more likely due to rapid use of diphenylamine in cattle's drug, in milk processing plants and packaging materials.

2.3.4. Toxicological consequences of pesticides

2.3.4.1. Organophosphates

Chlorpyrifos

Chemical Structure:

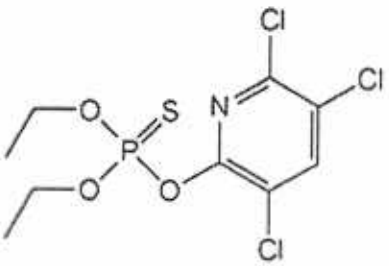


Table 2.3.4.1.1. Physical and chemical features of Chloropyrifos
(Data adapted from: Chloropyriphos 232. WHO pesticide residue series 2, Chloropyriphos WHO pesticide residue series, Chlorpyrifos (ICSC), Chloropyriphos (PDS), Chlorpyrifos JMPR Evaluations, 1999)

Type	broad-spectrum organophosphate insecticide
Chemical Name	O,O-Diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate
Formula	$C_9H_{11}Cl_3NO_3PS$
Molecular weight	350.6 g
Melting point	42-43.5 oC
Vapour pressure	1.87×10^{-5} mm Hg (at 25°C); 8.87×10^{-5} mm Hg (at 35°C)
Solubility	acetone, benzene, chloroform, methanol; iso-octane; slightly soluble (0.4 mg kg^{-1}) in water at 2.0 mg L^{-1}
Half Life	11-180 days in soil, 35-78 days in water, 10-14 days in plants, 4-7 days in animal tissues
Toxic dose	Rat= 0.03 mg per kg per day , Dog: 0.01 mg/kg/day , Man: 0.014 mg/kg/day orally for 1 month, Acceptable Daily Intake For Man is 0 - 0.0015 mg per kg of body-weight
Maximum Tolerable limits in plants, vegetables	Chinese cabbage, grapes, kale, apples is 1 mg kg^{-1} Carrots, pears, tomatoes is 0.5 mg kg^{-1} eggplants, peppers, raspberries is 0.2 mg kg^{-1} Lettuce, rice (in husk), sugar beet is 0.1 mg kg^{-1} Celery, cottonseed, cottonseed oil, mushrooms, onions is 0.05 mg kg^{-1}. Cauliflower, milk (fat basis), potatoes, red cabbage is 0.01 mg kg^{-1}. Fat of meat of cattle is 2 mg kg^{-1} Fat of meat of sheep, poultry, beans, is 2 mg kg^{-1}

Uses

Chloropyrifos is widely used around the world as an insecticide over agricultural land, pastures, rangelands, and forests. In addition to that chloropyrifos is successfully applied around the herds to protect cattle from pest attack.

Mode of Action:

The basic action of Chlorpyrifos is that it inhibits the enzyme cholinesterase in brain and blood, but the severity is different in different animals. (Smith, 1966).

Toxicity

Chloropyrifos dietary exposure in cattle milk reached 0.01 mg kg^{-1} when fed on a diet containing 30 mg chloropyrifos per mg per day. According to CCPR report 0.01 mg kg^{-1} is the maximum residue limit of chloropyrifos in cattle fat or milk (Smith, 1968). When dietary intake of chloropyrifos either by human or cattle is more than MRL i.e 0.03 mg kg^{-1} it causes inhibition of plasma cholinesterase which causes depression in human, and no inhibition of plasma ChE is noted at 0.1 mg kg^{-1} of chloropyrifos in diet. Acute toxicity of chloropyrifos via oral, dermal, inhalation and ingestion is given below.

Table 2.3.4.1.2. Chloropyrifos is categorized in the following classes according to USEPA Manual (2009)

	Toxicity classification of chloropyrifos			
	High	**Moderate**	**Low**	**Very low**
Acute Oral LD_{50}	≤50mg/kg	>50-500 mg/kg	>500-5000 mg/kg	>5000 mg/kg
Inhalation LC_{50}	≤ 0.05mg/L (aerosol)	> 0.05-0.5 mg/L	> 0.05-2.0 mg/L	>2.0 mg/L (dust)
Dermal LD_{50}	≤ 200 mg / kg	> 200-2000 mg / kg	> 2000 - 5000 mg / kg	>5000 mg / kg
Primary Eye Irritation	irreversible destruction of eye tissue or corneal involvement / irritation persisting for more than 21 days	Corneal involvement or other eye irritation clearing in 8 to 21 days	Corneal involvement or other eye irritation clearing in 7 days or less	Minimal effects clearing in less than 24 hours
Primary skin irritation	Corrosive eye tissue destruction and/or scarring)	severe erythema odema upto 72 hrs	moderate erythema upto 72 hrs	Mild or slight irritation at 72 hours

Eco Toxicity

Chlorpyrifos is highly toxic to all types of aquatic life in whose bodies it accumulates. It is not very soluable in water but strongly adsorbs to soil particles. The soil carrying chloropyrifos ultimately finds it's way to water bodies and settles at the bottom where it poses a persistent hazard to aquatic life living at the bottom (Smith et al., 1991). It is also harmful to honeybees and birds. The harmful effects of chloropyrifos increase at low temperatures.

Degradation of Chlorpyrophos

Degradation in soils

Chloropyrifos persists in the soil for a varying period ranging from 2 weeks to 1 year and even upto 4 years (Wright et al., 1991). The great variation is due to the difference in the type of soil, temperature, PH etc. (Gilani, et al., 2010). Degradation of chloropyriphos in soil is a hydrolytic process resulting in the formation of 3,5,6 trichloro 2 pyridinol (Racke et al.,1990)

Degradation in Water

When chlorpyrifos dissolved in water is exposed to sunlight, UV rays hydrolyse it very quickly at pH above 8. The initial product is 3,5,6-trichloro-2-pyridinol which is subsequently converted to diols, triols and fragmentary products. (Smith, 1968).

Degradation in Mammals

In mammals after oral administration, it is quickly absorbed, metabolized and excreted by desethylation to Cl 3,5,6-trichloro-2-pyridyl phosphate (75-80%), 3,5,6-trichloro-2-

pyridinol (15-20%), pyridyl phosphate and pyridinol. Very minute quantities of unchanged chlorpyriphos are excreted (Carlile, 2006). Small amount of chlopyrifos can be found in fatty tissue for a half life of 3 days The major route of excretion is urine and faeces. Small quantity of < 0.3 ppm, are found in liver, kidney and blood. (Gutemann, 1968).

Degradation in Plants

Degradation in plants occurs by oxidation and/or hydrolysis resulting in the formation of des-mono-ethylchloropyrifos, desethyl chlorpyrifos, 3,5,6-trichloro-2-pyridinol and further degradation products (Smith, 1967). Chlorpyrifos evaporates from leaves upto 80% within 48 hours and therefore residues do not build up in plants after repeated applications. Very minute quantity of residues can be found in the form of chlorpyrifos and 3,5,6-trichloro-2-pyridinol (Smith, 1966).

Studies

Coulston et al. (1972) studied the effect of oral dose of chloropyrifos on adult male rats. No effect on behavior, hematology, urinalysis and biochemical changes in blood was noted in any of the male adult. However depression of plasma cholinesterase was noted in those male adults who had 0.03 mg of chloropyrifos.

In another study JMPR (1972) reported "no evidence of neurotoxicity, teratogenecity, reproductive abnormality or cataractogenicity in male adults whereas increase in neonatal mortality was observed when chloropyrifos was given at 1 mg $kg^{-1}day^{-1}$. Residues of chlorpyriphos were predominately found in the fatty tissues, and residues of 3,5,6-

trichloro-2-pyridinol were in the kidney and liver tissues. No residue of the oxygen analogue was detected in any tissue." Chlorpyriphos is more potent than other organophosphates.

Salas et al (2003) carried out a study on residue of 13 organophosphorus pesticides in pasteurized dairy milk in Mexico. These pesticides are commonly used over the crops and fodder. They found that about 39.6% of the pasteurized milk samples contained OP pesticide residues. Eight pasteurized milk samples contained residues of dichlorvos (five samples), phorate, chlorpyrifos, and chlorfenvinphos (one sample) more than their maximum residue limits (MRL). However some pasteurized milk samples contains the residue of 13 OP pesticides ranged from 0.0051 to 0.0203 mg kg^{-1} and remained with their maximum residue limits. Gazotti et al. (2009) studied the presence of chloropyrifos in dairy milk samples in Italy. They reported that chloropyrifos was present in traces in some milk samples whereas it was completely absent in other milk samples.

In another study Ciscato et al (2002) reported traces of "organophosphates, carbamates, pyrethroids, herbicides and fungicides in cow's milk samples" where endosulphan and HCH were absent in other milk samples in dairy cow's of Brazil.

Profenofos

Chemical Structure

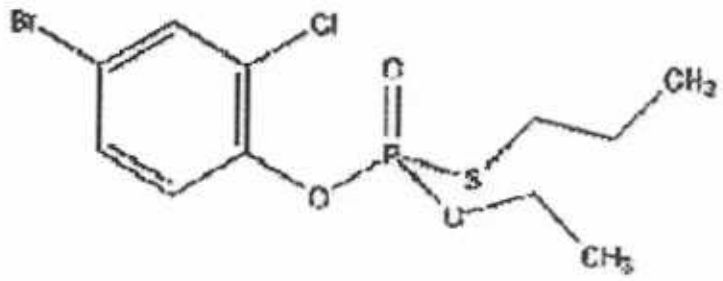


Table 2.3.4.1.3. Physical and chemical features of Profenofos (adapted from Quest, 1990; Material Safety Data Sheet, 2010).

Type	Organophosphorus insecticide
Chemical Name	O-4-bromo-2-chlorophenylO-ethyl S-propyl phosphorothioate
Formula	$C_{11}H_{15}BrClO_3PS$
Molecular weight	373.6 g
Melting point	Not applicable
Vapour pressure	2.53 mpa at 25°C
Solubility	
Half Life	Soil is 7 days Water not mentioned
Toxic dose	Mouse, 30 mg kg^{-1} or 5.8 mg kg^{-1} bw day^{-1} in females. Rat, 20 mg kg^{-1}, 1.0 mg kg^{-1} bw day^{-1} (reproduction). Rat, 100 mg kg^{-1}, 5.7 mg kg^{-1} bw day^{-1} in males (long-term study).
Maximum Tolerable limits in humans	0-0.01 mg/kg body weight

Uses

An insecticide used on a "wide variety of crops to control many pests but mainly tobacco budworm, cotton bollworm, armyworm, cotton aphid, whiteflies, spider mites, plant

bugs, and fleahoppers on cotton, maize, sugarcane, soya beans, potatoes, vegetables, tobacco and other crops". About 85% of all profenofos is used on worms out of which one third is used at maximum rate (EPA, 2000).

Mode of action

Profenofos inhibits acetylcholinesterase. It acts on worms and insects on skin contact and stomach after ingestion. Profenofos exhibits translaminar effect and ovicidal properties (Leader and Casida, 1982).

Toxicity

In human beings the acceptable daily intake is 0 - 0.03 mg/kg bw day^{-1} , oral acute LD_{50} for rats is 350 mg kg^{-1} and for rabbits is 700 mg kg^{-1} while a cut reference dose is 1.0 mg/kg bw day^{-1} (JMPR 2007). According to WHO (1999) report profenofos is considered as moderately hazardous. Oral poisoning of profenofos usually occurs accidentally when profenofos is placed in an unlabelled container and taken as a drink. Nevertheless it's dietary intake when greater than 0.01 mg kg^{-1} day^{-1} may cause severe health hazards in human and animals. Dermal toxicity occurs when profenofos is in contact with skin accidentally because of leakage or spillage. Profenofos penetrates from the skin into the body. Toxicity symptoms are "excessive salivation, sweating, rhinorrhea and tearing, muscle twitching, weakness, tremor, headache, dizziness, nausea, vomiting, abdominal cramps, diarrhea, respiratory depression, tightness in chest, and cholinesterase inhibition. No teratogenic or mutagenic effects are known in animals (WHO, 1990; JMPR, 2007)." In a research in Multan, Pakistan, 6 spraymen between the age group of 20 to 25 years, were monitored for 4 days for the effects of profenofos on blood cholinesterase levels

while spraying on cotton plant. It was found that there was inhibition of blood cholinesterase activity. (Loosli, 1989).

Eco toxicity

The ecotoxicity of profenophos for birds is high with acute LD50 of 70-200 mg kg^{-1} .For fish it is also high with acute 96 hour LC_{50} of 0.08 mg l^{-1} but for aquatic invertabrates it is moderate. However for honeybees the acute 48 hour LD_{50} is high which is 0.095 ug kg^{-1}. (PPDP, 2004)

Degradation of Profenophos

Degradation in soil

Profenofos in it's original form is not persistent in alkaline and neutral soils. It dissipates slowly and undergoes initially hydrolysis and then aerobic and anaerobic degradation to form it's major metabolite, 4-bromo-2-chlorophenol, which persists in the environment while the minor one is O-ethyl-S-propyl phosphorthioate, whose persistence is not known (Material Safety Data Sheet, 2010).

Degradation in water

Profenofos is not usually found in undergroud waters because it does not penetrate in the soil, however it may be found on surface waters due to surface run-off or during spraying and because of this it may gain entry to groundwaters (MSDS, 2006). Profenofos is non persistent under alkaline conditions and degraded rapidly through the processes of hydrolysis. Under acidic conditions degradation of profenofos is extremely slow. Nevertheless, the mode of degradation under acidic and alkaline conditions remained same with slight difference in the pathways under both conditions. The degradation

product such as -bromo-2-chlorophenol and O-ethyl-S-propyl phosphorthioate remained same under alkaline and acidic conditions (Material Safety Data Sheet, 2010).

Degradation in mammals

Degradation in mammals takes place by 2 pathways. The major one produces 4-bromo-2-chlorophenol by depropylation, desulfuration, and phenyl-ester bond cleavage while the minor also produces the same end product through de-ethylation and phenyl-ester bond cleavage. Finally there is conjugation with glucuronic and sulfuric acids. The end metabolites and a minute quantity of the parent compound is excreted in faeces while no parent compound is excreted in urine (Ifflaender, 1974).

Studies

For the removal of pesticides it has been suggested that zero valant metals like iron, tin and zinc in combination with ultrasonic irradiation cause the reduction and hence degradation of carbon tetrachloride, pentachlorophenol, nitrobenzene, hexachlorocyclo hexanes, azo dyes and a number of pesticidies like imidacloprid (more than 90%) and thiamethoxam. The pH should be 2.0 and reaction time 30 min (Renata et al., 2008). A research was conducted in Minas Gerais, Brazil in which solid phase microextraction (SPME) was used for the extraction of residual organophosphorus pesticides in whole milk. The results suggest that Organophsosphorus pesticides used in dairy farming contaminated dairy milk and the residues are not removed by treating the milk by boiling (Cardeal and Dias, 2006)

Research has been done out to find out the effect of milk processing on the levels of pesticides and their metabolites in milk. It has been shown that heat treatment used for producing dry milk and pasteurized milk destroyed some of the residues present. The

amount of residue destroyed depends upon the processing treatment and nature of the insecticide residue. But it has been also found that chlorinated hydrocarbons are most resistant to heat treatment and once they get into milk they are difficult to remove (Liaska, 1968). A study showed that applying heat to milk as in boiling or pasteurization and lactic acid fermentation as in yoghurt preparation decrease the concentration of most of the Organophosphates pesticides (Li and Zhao, 2010).

A study was carried out on 15 samples of dairy milk in and around Delhi. It was found out that pesticide residue level was more than the permissible limits given by WHO/FAO joint expert committee on food additives. The levels were found ranging from 0.022 to 0.166 $\mu g\ gm^{-1}$ for HCH and from 0.042 to 0.382 $\mu g\ gm^{-1}$ for DDT (Mukherjee and Gopal, 1993). Srivasta et al. (2008) showed that in Allahabad, India, bovine milk samples were studied for organophosphorus and organo chlorine pesticides. The results showed that BHC was found in 75% of the samples, methyl parathion in 37.5% of the samples, dieldrin and DDE in 12.5% of samples each. The mean levels were higher than the acceptable daily intake level as given by Prevention of Food Adulteration Act India, 1992. The values were higher in rural areas than in urban. Research studies conducted in Nepal have showed that over 60% of the farmers using pesticides over 5 years wait less than two weeks after spraying before harvesting the crop. Therefore pesticide misuse has to be checked in Nepal just like all the other Asian countries (Palikhe, 2002)

2.3.4.2.Pyrethroids

Cypermethrin

Chemical Structure

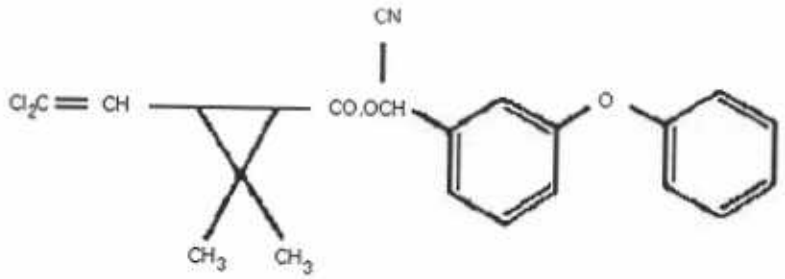


Table 2.3.4.2.1. Physical and chemical features of Cypermethrin

(Data from Kollman and Segawa, 1995, Walker and Keith, 1992, USDA Agricultural Research Service, 1995, Kidd and James, 1991)

Type	Synthetic Pyrethroid	
Chemical Name	(RS)-cyano(3phenoxyphenyl)methyl(1RS)-*cis-trans*-3-(2,2-dichloroethenyl)-2,2 dimethyl-s s cyclopropane carboxylate	
Formula	$C_{22}H_{19}O_3NC_{l2}$	
Molecular weight	416.3.	
Melting point		
Vapour pressure	1.3x10-9 mmHg(at 200° C)	
Solubility	4 ppb (200°C)	
Half Life	Aerobic 6-20 days Anerobic <14 days	
Toxic dose	Rat LD_{50} LD_{50} Rabbit LD_{50}	247 mg/kg (males) 309 mg/kg (females) > 2460 mg/kg
Maximum Tolerable limits in plants, vegetables		

Uses

Cypermethrin is widely used throughout the world as an insecticide over agricultural land, pastures, rangelands, and forests. It is also used for the treatment of ectoparasites of cattle, sheep and poultry.

Mode Of Action

Cypermethrin mainly acts on the nervous system by direct contact but it can also act as a stomach poison on ingestion. It's mode of action is to keep the sodium channel of the nerve membranes open for a very long period by altering the voltage. As a result of this the permeability of nerve membranes for sodium is increased leading to repetitive nerve impulses. ATPase enzyme provides energy for the movement of ions against a concentration gradient and this action is especially pronounced at sites of oxygen transport like skin and membranes in fish. Cypermethrin inhibits ATPase so active transport of ions cannot take place due to which fish and other aquatic animals are severely effected. (Jin and Webster, 1998; Vijverberg and Van den Bercken, 1990).

Toxicity

Cypermethrin maximum residue limits for dietary exposure in cattle milk is 0.05 mg kg^{-1} when fed on a diet containing 50 to 100 mg cypermethrin per mg per day while the acceptable daily intake is 0.02 mg kg^{-1} (CODEX alimentarius FAO/WHO, 2010). When dietary intake of cypermethrin by humans is more than ADI, phenoxybenzoic acid and cyclopropanecarboxylic acid derivates are formed as conjugates. Acute toxicity of cypermethrin is via oral, dermal, inhalation and ingestion is given in Table 2.3.4.3.2 (Kaufman et al., 1981; USDA, 1995).

The oral toxic dose LD50 is 1085 mg kg^{-1}.The signs of acute toxicity are "loss of motor control, tremors, decreased activity, urinary incontinence, incoordination, increased sensitivity to sound and convulsions."

Dermal LD50 = >2000 mg kg^{-1} may produce numbness, burning and tingling

Inhalation LD50 of 12.35mg/l/hr causes "tremors, decreased activity, urinary incontinence, incoordination, increased sensitivity to sound and convulsions" (Material Safety Data Sheet, 2011. www.cdms.net/LDat/mp6S8002.pdf).

Table 2.3.4.2.2 Cypermethrin is categorized in the following classes according to USEPA Manual (2009).

	Toxicity classification of chloropyrifos			
	High	**Moderate**	**Low**	**Very low**
Acute Oral LD_{50}	≤ 50 mg / kg	> 50 – 500 mg / kg	> 500 – 5000 mg / kg	> 5000 mg / kg
Inhalation LC_{50}	≤ 0.05 mg /L (aerosol)	> 0.05 - 0.5 mg / L	(> 0.05 - 2.0 mg / L	> 2.0 mg / L (dust)
Dermal LD_{50}	≤ 200 mg / kg	> 200 - 2000 mg / kg	> 2000 - 5000 mg / kg	> 5000 mg / kg
Primary Eye Irritation	"irreversible eye destruction or corneal involvement or irritation persisting for more than 21 days"	"Corneal involvement or other eye irritation clearing in 8 to 21 days"	"Corneal involvement or other eye irritation clearing in 7 days or less"	"Minimal effects clearing in less than 24 hours"
Primary skin irritation	"Corrosive tissue destruction into the dermis and/or scarring"	Severe erythema odema upto 72 hrs	moderate erythema upto 72 hrs	Mild or slight irritation at 72 hours

Eco Toxicity

Synthetic pyrethroids are extremely effective against insects because insects have a low metabolic rate, but are relatively safe to mammals and birds. However they are very toxic to aquatic organisms even in minute doses of <1 µg/L (Siegfried, 1993). Because it has more affinity for body fats and low for water therefore when it is present in an aquatic environment it either binds to soil particles or to fats of aquatic animals. It's ecotoxicity

to fish is lower in those waters where there is more soil particles and particulate matter because it preferentially binds to them and toxicity to fish will be more when the water is deficient in sediment and particulate (Bacci, 1987; Muir et al., 1985).

Degradation of Cypermethrin

Degradation in soil

Cypermethrin binds to soil surface quickly and does not move out from soil into the surrounding water. But as degradation occurs, the products particularly PBA and DCVA move out through soil. Cypermethrin degrades more quickly in the presence of microbes, aerobic conditions, natural soils, sandy soils. Cypermethrin degrades more slowly and therefore persists in the soil under anaerobic conditions, reduced microbial activity, waterlogged conditions, sterilized soils, more clay content in soils, more organic matter and in sterilized soils (Kaufman, 1981; Walker and Keith, 1992; USDA ARS, 1995; Chapman, 1981).

There are two major methods of degradation of cypermethrin in soil and water and these are Hydrolysis and Photolysis. The main route is Hydrolysis and leads to the formation of 3-phenoxybenzoic acid (PBA) and cyclopropanecarboxylic acid derivatives, principally, 3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylic acid (DCVA).

Photolysis occurs rapidly to many metabolites, with half-lives of 8-16 days, most important of which are PBA and DCVA. These break down to CO_2 under aerobic conditions. (Sakata, 1986; Kaufman, 1981; Hall et al., 1981; Walker and Keith, 1992).

Degradation in Water

In soil and water, the major metabolites are PBA and DCVA via hydrolysis and photolysis, with the impurity 3-phenoxybenzaldehyde as a minor by product in water Cypermethrin is very less soluable in water, only upto 4 ppb at 200°C. It quickly moves away from water particles to soil, sediment and suspended particulates.Cypermethrin degrades quickly in polluted waters, at an alkaline pH and presence of sunlight. But it remains stable at normal environmental temperatures, neutral and acidic pH, darkness, sterile conditions where it's half-life ranges from 2 to more than 3 months. Cypermethrin, bound to soil, may be carried away along with rain and irrigation water to nearby waters, but it does pose any hazard to aquatic life because of it's affinity to soil particles (Walker and Keith, 1992; Takahashi et al., 1985; Agnihorti, 1986).

Degradation in mammals

The major metabolites of cypermethrin in mammals are PBA and 4'-hydroxy-3-phenoxybenzoic acid but it is mostly excreted in its original form. It has high affinity for fats but it is not significantly stored in fatty tissues and is quickly excreted within a week. (Leahey, 1985)

Degradation in plants

In plants the major metabolic pathway is ester cleavage which produces α-cyano-3-phenoxybenzyl alcohol. The minor pathway is hydroxylation but it is less common (Leahey, 1985).

Studies

In one study by Chen et al. (1997), the results showed that cypermethrin residues were less than 19 ppb in the milk samples when cows were given a 5 ppm daily dose. Also, cypermethrin and its metabolites could not be detected in any tissue samples except for fat samples which showed up to 125 ppb of cypermethrin. Almost half of it was excreted in urine and half in faeces.

A study was carried out in Sao Palou, Brazil, especially to determine the concentration of cypermethrin in milk after it was given to cows in a formulation for veterinary treatment for the elimination of parasites. The formulation contained 50 g L^{-1} of active ingredient in it. The results showed that cypermethrin was found in milk upto 0.168 mg kg^{-1} 24 h after treatment. 16 times higher levels (than the MRL 0.010 mg kg^{-1}) were detected 15 and 24 h after treatment and 7 times higher levels, 11 days after treatment. However it was not detectable 17 days after treatment (Sassine and Maura, 2004). Another study was conducted in India in which 92 samples of milk were analyzed in Ludhiana. These samples were found to contain DDT and Lindane in quantities above the maximum residue limit (MRL), however organophosphates or synthetic pyrethroid were not found in any of the milk and butter samples where the minimum detection limit was of 0.01 mg kg^{-1}(Battu et al., 2004). A single injection of pyrethroid insecticides flumethrin, deltamethrin, cypermethrin and cyhalothrin was given to dairy cows in recommended doses and their presence was detected in milk and blood of 10 cows in Brazil. Milk and blood samples were collected after every week for a month. "The highest residues in milk were found on day 28 for flumethrin and day 1 for deltamethrin, cypermethrin and cyhalothrin (Bissacot and Vassilieff, 1997)."

The USDA's Pesticide Data Program (PDP) tests food items for pesticides. According to USDA, reports only 15% of the samples were positive in low concentrations in 1996, 1997, and 1998 and none had a pyrethroid residue. However in 2004, 100% of the 739 milk samples showed residues, and almost one fourth of the samples had a synthetic pyrethroid residue. It was analyzed that this was due to the fact that the "methods used to test milk in 2004 were 100-times more sensitive in picking up Diphenylamine residues, and 17-times more sensitive in detecting Dicchloro-diflouro ethane (DDE) and endosulfan than the methods used in 1996-1998". DDE is extensively present in the atmosphere because of the extensive use of DDT in the past and therefore it is found in the body fats of all animals and human through out the world. Diphenylamine is used as an apple growth regulator but its main entry into milk is probably through some other routes like "drug injections, rubber and plastic products used for burning on dairy farms or in milk processing plants, or ingredients used in milk cartons and packaging (FAQ, 2010)."

2.3.4.3. Nicotinnoids

Imidacloprid

Chemical structure:

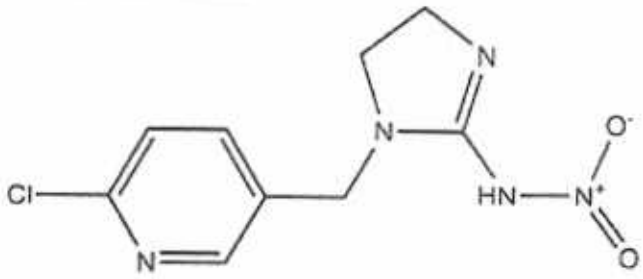


Table 2.3.4.3.1 Physical and chemical features of Chloropyrifos (adapted from Sarkar et al., 2001).

Type	Neonicotinoid insecticides or chloronicotinyl insecticide are synthetic derivatives of nicotine
Chemical Name	1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine
Formula	$C_9H_{10}ClN_5O_2$
Molecular weight	255.7 g
Melting point	
Vapour pressure	7: 3 x 10-12 mmHg at 20 °C
Solubility	0.61 g $l.^{-1}$ at 20 °C, 514 mg $l.^{-1}$ (20°C at pH 7)
Half Life	Hydrolysis half life is > 30 days at 25°C at PH 7 Aqueous photolysis half life < 1 hour at 24°C at pH 7 Anaerobic half life is 997 days, Aerobic half life is 99 days Soil photolysis half life is 38.9 days, Field Dissipation half life is 26.5-229 days, Half life in unamended soil is 40 days Half life in soil amended with fertilizers is 124 days Half life in non-agricultural soil is 188-997 days Half life in cropped soils is 69 days.
Toxic dose	0.057 mg $kg^{-1}day^{-1}$ based on chronic toxicity 5.7 mg $kg^{-1}day^{-1}$ no chronic toxicity 16.9 mg kg^{-1} per 31 day low adverse effect
Maximum Tolerable limits in plants, vegetables	

Uses:

"Imidacloprid is used to kill sucking insects, some chewing insects including termites, soil insects, and fleas on pets. Imidacloprid may be applied to structures, crops, soil, and as a seed treatment" (Fossin, 2006).

Mode of action:

Imidacloprid is a contact poison and stomach poison. "It is a systemic insecticide that translocates rapidly through plant tissues" (Tomlin, 2006). Imidacloprid binds irreversibly to post-synaptic acetylcholine receptors in the nervous system and blocks the binding of acetylcholine. Acetylcholine accumulates at the receptor leading to paralysis and death of insect (Ware and Whitecare, 2004) Because of binding of imidacloprid to receptors, nerve impulses are discharged rapidly initially and then stop altogether (Matsuda and Sattelle, 2005). "However, the binding affinity of imidacloprid for mammalian nicotinic receptors is much less than that of insect nicotinic receptors (Schroeder and Flattum, 1984)." The blood-brain barrier in mammals blocks the passage of imidacloprid to the central nervous system (Imidacloprid Technical Fact Sheet, 2010). Acetylcholinesterase cannot break down the pesticide. (Buckingham et al., 1997).

Toxicity:

Route	Toxicity	USEPA Category	LD50 for rat
Ingestion	Moderate	II	130-170 mg kg^{-1}
Dermal	Low	IV	
Inhalation	Low	IV	5323 mg per m^3
Aerosol	High	I	69 mg per m^3

Source:- USEPA, 2009, Thyssen and Machemer, 1999, USEPALRM, 2009

It is placed in group E, non-carcinogenic for humans, by US EPA (Thyssen, 1999).

Signs of Toxicity in animals with increasing doses are salivation, vomiting, lethargy, diarrhea, muscle weakness including respiratory muscles, ataxia, twitching, cramps, convulsions, uncoordinated gait, tremors, and reduced activity. On contact with skin it can cause hypersensitivity reactions. In acute cases symptoms appear rapidly within 15 minutes and disappear rapidly, within 24 hours. However if lethal doses are given, death can occur within 24 hours. But neurotoxic effects are not long lasting and delayed effects also do not appear (Hovda and Hooser, 2002; Sheets, 2002; Wismer, 2004).

Olefin and nitrosamine are two of it's metabolites which appear in plants treated with imidacloprid and these are more potent than the parent compound. The guanidine metabolite has a high mammalian toxicity but does not possess insecticidal properties. Chronic toxicity of imidacloprid causes reproductive and mutagenic effects at relatively high doses (Nauen, 1998; Tomizawa and Casida, 1999).

Ecotoxicity

Imidacloprid has a low vapor pressure due to which it has very low ability to disperse from water into the air over a large area. LD_{50} for trout is 211 which shows that toxicity to fish is low. "Many non-target beneficial arthropods such as honeybees, parasitic wasps, and predaceous ground beetles may be adversely affected by sublethal doses of the insecticide, but the effects depend on method of application and route of intake (Cox et al., 1997)."

Degradation of Imidacloprid:

Degradation in Soil

The adsorption coefficient in soil is low 132-310 due to which it has tendency to stay in soil. The affinity for soil increases if there is more organic matter in the soil, presence of microbes, lowering of concentration. Sorbtion decreases at high soil concentrations of imidacloprid, sandy loam soil, more dissolved organic carbon in calcareous soil (Cox, 1997; Flores, 2002).

Imidacloprid is degraded in soil by the action of light to 6-chloronicotinic acid, cyclic ureas, olefinic cyclic nitroguanidine, a cyclic guanidine, and its nitroso and nitro derivatives. After 3-4 months, metabolites are 2% of the radiocarbon label (Roberts and Hutson, 1999).

Degradation in Water

"Imidacloprid is broken down in water by photolysis. Imidacloprid is stable to hydrolysis in acidic or neutral conditions, but hydrolysis increases with increasing alkaline pH and temperature. The primary degradation products resulting from aqueous photolysis are the same as in soil (Moza et al., 1998)". The combination of low soil adsorbtion coefficient (132–310) and high water solubility of 514 mg kg^{-1} shows that it can leach to groundwater. The leaching potential is increased by the presence of dissolved organic carbon, presence of carriers and surfactants, soil compaction and rainfall (Felsot, 1998). Imidacloprid has a low vapor pressure 1.0×10^{-7} mmHg and low Henry's law constant of 6.5×10^{-11} atm. m^3 $mole^{-1}$ which suggests that it has low volatility from water and does not disperse in air over a large area (Fossin, 2006).

Degradation in Mammals

Mammals metabolize imidacloprid primarily in the liver by two major pathways. In the major pathway, imidacloprid is broken down to 6-chloronicotinic acid and imidazolidine. Imidazolidine is excreted in the urine, and 6-chloronicotinic acid undergoes further metabolism via glutathione conjugation to form mercaptonicotinic acid and a hippuric acid. In the second major pathway there is hydroxylation of the imidazolidine ring to 5-hydroxy and olefin derivatives. 80% of the metabolites are excreted in faeces and 20% in urine. 90% is excreted in first 24 hours and 96% in first 48 hours (INCHEM, 2001)

Degradation in Plants

Imidacloprid is absorbed from soil by the plant and can be found in all plant tissues like leaves, vascular fluids, and pollen (Tomlin, 2006). The main metabolites in plants are monohydroxy metabolite, imidacloprid guanidine, imidacloprid olefin, monoglucoside of 6-chloropicolyl alcohol (Miles, 1993)

Studies

The United States Department of Agriculture (USDA) Pesticide Data Program studied and published in 2006, the presence of imidacloprid residues in food. Imidacloprid was detected in many fresh and processed fruits and vegetables. "It was detected in over 80% of all bananas tested, 76% of cauliflower, and 72% of spinach samples. In all cases, however, the levels detected were below the U.S. EPA's tolerance levels".

Chapter 3

MATERIAL & METHODS

3.0. MATERIAL AND METHODS

3.1. Map and Description of the area

Peshawar District situated on the Iranian Plateau, "extends over 50 kms (31 mi) north south and over 30 kms (19 mi) east west. It is situated at an altitude of 359 m (1,138 ft) above sea level. The Peshawar valley is nearly circular, extending from the Indus to the Khyber Hills. More than 99% of the city's population is Muslim, mostly Sunnis with Shias and Ahmadis as the minority. The sub-soil stratum is composed of gravels, boulders, and sands below which is confined water bearing aquifer at more than 400 feet (120 m). Peshawar has very hot summers and mild winters. The city has a population of nearly 40,00,000 with a current growth rate of 3.3% which is higher than that of the rest of the country. The estimates of 2002 show doubling of city's population from 1.1 million in 1981 to 2.242 million in 2002 due to a growth rate of 3.56% (http.//.www.maplindia.com)."

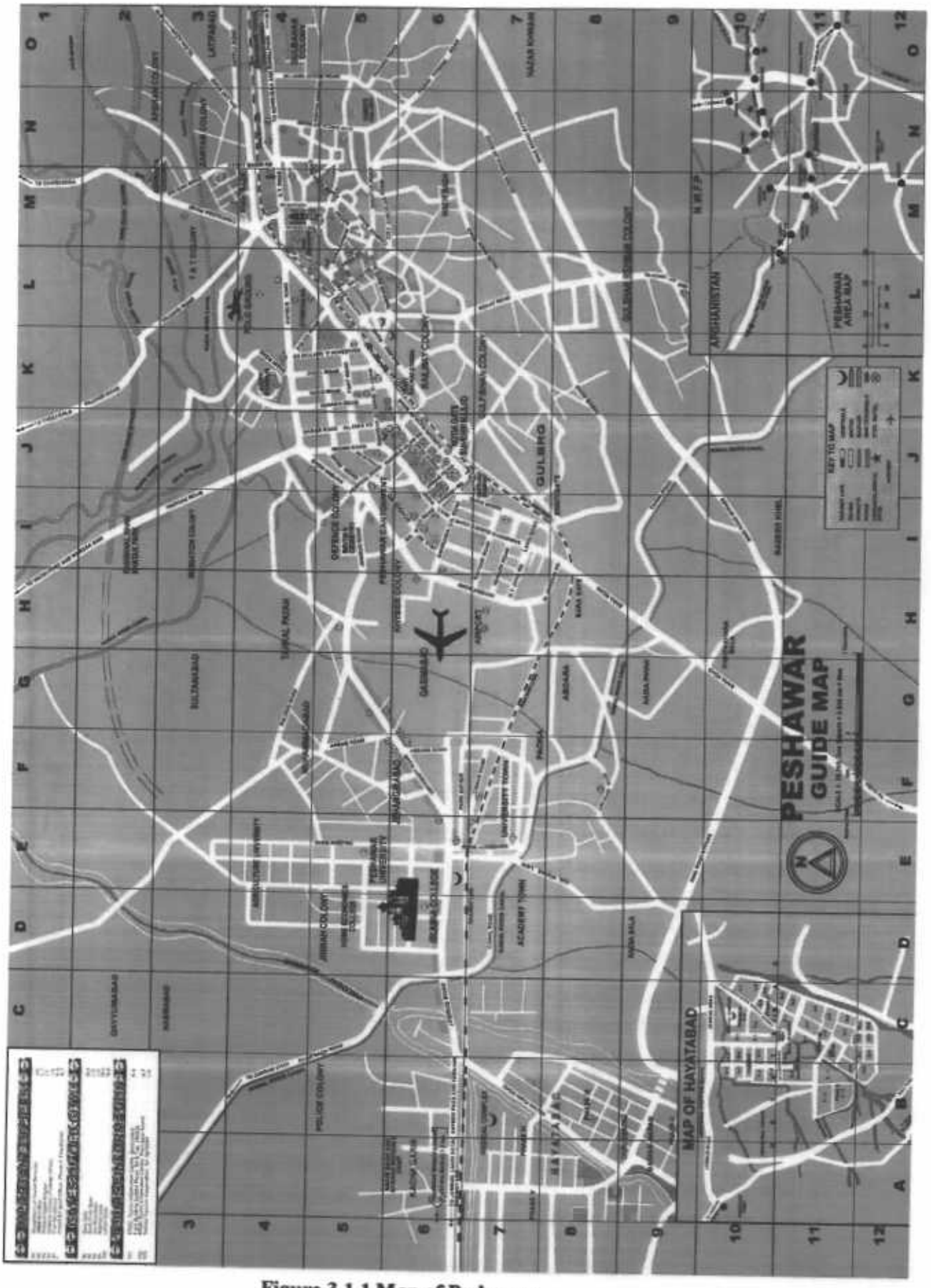


Figure 3.1.1 Map of Peshawar

3.1.1. General Dairy farm conditions

Dairy farming system in Peshawar is mixed farming system, urban and peri-urban dairy farming. Mixed farming is commonly used in rural areas where small numbers of cattle (up to 10 in numbers) are kept in herds. Around 57% of cattle are kept in this system (Sarwar et al., 2002). Cattle are fed on fodder, crop residue, weeds and agro industrial waste products after harvesting. Feeding and grazing requirement of cattle is low and this is a resource poor system which needs further modification. Urban and peri-urban dairy farming is a system which is developed in and around Peshawar city and in towns in order to meet the demand for milk in the city. Mainly cattle are kept in small herds located within the centre of towns. The cattle are fed on agro industrial by- products such as cotton khal, sugarcane khal, maize khal or wheat grains available commercially. Around 32 to 47% of cattle are kept in herds in this system (Livestock Census Report, 1996). Cattle are taken out for grazing to the open field surrounding the dairy farms. Sugarcane khal is obtained from sugarcane industry of Charsadda and Mardan. Wheat bran is purchased from flour industry whereas cotton khal is obtained from Punjab whereas fresh grasses are available locally. All cattle in urban and peri-urban dairy farms are on minimum grazing whereas in rural farms they are on high grazing. Urban and peri-urban farms are located either in the densely populated towns, near highways or industrial processing units hence cattle in the farm are vulnerable to environmental pollution. The size of the dairy farms is not more than 3 kanal and water and food containers are corrosive and unhygienic.

3.2. Reagents and Glassware

The reagents used for heavy metal analysis were 1M HNO_3, HCl, H_2O_2, salts of Pb, Cu, Co, Cr,Cd & Ni, distilled water and Whatmann filter paper no. 42. All reagents used were analytical grade of Merck-Germany. The reagents used for pesticides were acetonotrile, QuEChERs tube 1, which is a polypropylene centrifuge tube named ECMSSC50CT containing 6 grams anhydrous $MgSO_4$ and 1.0 gram of NaCl, another QuEChERs dispersive solid-phase cleanup tube: UCT ECMPSC1815CT which is a 15mL centrifuge tube with 900mg anhydrous magnesium sulfate, 300mg PSA & 150mg endcapped C18. QuEChERs tubes were purchased from United Chemists Bristol USA.

The glass ware used were glass beakers, pipettes, burettes, conical flask, glass vials, gcms filtration tubes, tripod stand, hot plate and plastic bottles.

3.3. Sampling

3..3.1. Sampling feed/fodder samples

The fodder samples were collected from each of the dairy farm from which milk samples were collected. Since milk was collected from five farms (3 urban & 2 rural), as such five samples of each type of feed were collected thus making a total of 30 fodder samples.

3.3.2. Sampling milk samples

It was a cross-sectional study. The dairy farms were selected randomly. 50 samples of raw milk were collected from 3 dairy farms of urban localities and 2 of rural localities, 10

from each farm. Urban farms which were selected were located within the centre of densely populated towns in Gulbahar, Tehkal and University area. The rural farms were selected from the peri-urban villages of Achini and Sufaid Dheri.

Apart from this, 10 samples of pasteurized milk were taken for analysis and the brands which were studied were Haleeb, Nestle, Good milk and Olper. 10 samples of dry milk were also selected and the brands were Nido, Everyday, Milac and Tarang. About 100 mL sample of fresh cattle milk in triplicate was collected in 250 mL glass bottle at 3 p.m. The milk samples were placed in freezer until further analysis. The pasteurized and dry milk samples were also collected in triplicate and stored at -20°C for further analysis.

3.4. Sample preparation

3.4.1. Preparation of feed/fodder samples

3.4.1.1. Preparation of feed/fodder samples for heavy metals

Fodder samples were prepared for heavy metal analysis by wet digestion method as described by AOAC (1990). About 2 g of sugarcane khal, wheat bran, wheat grain, corn silage, cotton khal and mixed diet were placed in 30 mL glass beaker and were soaked in 10 mL of 1 M HNO_3 for 24 hrs. Thereafter the beaker was placed over the hotplate at 200°C for 15 to 20 minutes in order to ensure dryness. Once the red colored fumes of NO_2 disappeared, the beaker was cooled in the fume hood for 5 to 10 minutes. Thereafter, around 10 mL of aqua regea (HNO_3: HCl, 3:1) was poured in the beaker and was re-heated to ensure dryness. Finally the beaker was removed from the hotplate and was cooled for 5 to 10 minutes in the fumehood. Then the residue was dissolvedin 20 mL of .

distilled water, filtered through Whatmann filter paper No. 42 and stored in plastic bottles.

3.4.1.2. Preparation of feed/fodder samples for pesticides

Fodder samples for pesticides analysis were prepared by liquid extraction by QuEChERs method. QuEChERS stands for "Quick, Easy, Cheap, Effective, Rugged and Safe. QuEChERS is known as a multiclass, multiresidue method (MRM) for sample preparation and clean-up for the analysis of multiple pesticide residues in high water content (80-95%) matrices. It has been published as AOAC (Association of Official Analytical Chemists of US) method 2007.01 "Determination of Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate." It was developed and published by Anastassiades et al. (2003). The method is advantageous in that it gives high recoveries, accurate results, quick and low non-chlorinated solvent usage (QuEChErs Information Booklet, 2010)."

All cattle diets were dissolved in HNO_3 overnight prior to be used for extraction through QuEChERs method. After they were digested, 15 mL of acetonitrile was added to a 50ml polypropylene centrifuge tube named ECMSSC50CT containing 6 grams anhydrous $MgSO_4$ and 1.0 gram of NaCl and was shaken well. $MgSO_4$ was added to aid in partioning. Acetonitrile is used as a solvent because it is highly compatible with GC/MS and causes maximum extraction of the organic compounds without extracting lipophilic material. Organic solvent removes water during shaking and salts such as $MgSO_4$ and NaCl during shaking create an exothermic reaction with water, induce separation between water and acetonitrile and bind water to extract the pesticide analyte into acetonitrile

phase, thus causing high recovery of polar and water soluble pesticides from milk and cattle diet samples through GCMS (Anastassiades et al., 2003; Schenck and Wong, 2008;). "15gm homogenized sample was added to the centrifuge tube, shaken and then centrifuged for 1 minute at 3700 rpm. The supernatant was added to the dispersive solid-phase cleanup tube: UCT ECMPSC1815CT which is a 15mL centrifuge tube with 900mg anhydrous magnesium sulfate, 300mg PSA & 150mg endcapped C18. PSA {primary secondary amine} and carbon sorbents remove sugars and organic acids, plant pigments and planar compounds and sterols." It was then shaken for 1 minute, centrifuged again for 1 minute at 3700 rpm. The extract was filtered and then was placed in 15 mL air tight glass vial and was analyzed through GCMS.

The analysis of pesticides in food matrices using extraction by QueChERs and analysis by GCMS has been performed successfully by many researchers (Brown, 2005; Wong, 2007; Krumwiede et al., 2008; Sugitate and Kana, 2008; Duff and Voglino, 2010; Steiniger et al., 2010.)

3.4.2 Preparation of milk samples

3.4.2.1 Preparation of milk samples for heavy metal analysis

Milk samples were prepared for heavy metal analysis by wet digestion method followed by Atomic Absorption Spectrometry, as described by Ashton and Yousef (1966), Sadler (1982), Lauri et al. (2004), Cruz et al. (2009), Qin et al. (2009), Kinsara and Farid (2008), Kazi et al. (2009). Analytical methods for Atomic Absorbtion Spectrometry. Perkin Elmer (2000). About 1 mL of fresh and commercially available milk and 1 g of dry powder milk commercially available was placed in 100 mL glass beaker and diluted with

10 mL of 1 M HNO_3 and 3 mL of H_2O_2. The glass beaker was placed on hot plate at 200 °C for 15 to 20 minutes for dryness. The white ash was re-diluted with 10 mL of 1 M HNO_3 and was filtered through Whatmann No 42 and was placed in 20 mL glass bottles.

3.4.2.2. Preparation of milk samples for pesticide analysis

Milk samples were prepared for pesticide analysis using the same QuEChERs method as used for the extraction from fodder but the only difference was that the milk samples were not digested overnight in HNO_3. QuEChERs tubes were purchased from United Chemists Bristol USA.

3.5. Samples analysis

3.5.1. Analysis of feed/fodder samples for heavy metals

The fodder extract was analyzed for heavy metals by Atomic Absorption Spectrometer. Each standard solution was measured 3 times and the mean was plotted. A blank solution of distilled water was used to check accuracy of the standard solutions and it was run after every 10 samples. The metal content was calculated using the formula

$$\text{Concentration} = \frac{\text{Concentration of the element through AAS(ppm)} \times \text{sample volume}}{\text{Sample weight}}$$

mg L^{-1} or ppm

Atomic Absorption Spectrometer specifications

1. **Type:** A Analyst 700 single beam
2. **Light source:** Hollow cathode lamp to emit the spectrum of element of interest.

3. **Flame mode absorption cell** in which atoms of the sample are produced. However other types of cells can also be used like graphite furnace, MHS cell, MIAS cell, FIMS cell)
4. **Monochromator** for light dispersion
5. **Detector** which is a photomultiplier tube to measure the light intensity and produce an electric current.
6. **Electronic system** to measure the amount of light attenuation in the sample cell and convert it into actual sample readings to display it on the computer

Standard atomic absorption conditions for the elements under study

Elements	Wavelength (nm)	Energy	Flame type	Oxide Flow L/min	Fuel Flow L/min	Lamp Current	Slit Width	Linear Range mg/L	Characteristc concentration mg/L
Cu	324.8	68	Air/Acetylene	17.0	2.0	15	0.7H	5.0	0.077
Pb	283.3	46	Air/Acetylene	17.0	2.0	10	0.7H	20.0	o.45
Ni	232.0	46	Air/Acetylene	17.0	2.0	25	0.2H	0.14	2.0
Co	240.7	50	Air/Acetylene	17.0	2.0	30	0.2H	3.5	0.12
Cr	357.9	75	Air/Acetylene	17.0	2.0	25	0.7H	5.0	0.078
Cd	326.1/ 228.8		Air/Acetylene	S			o.7H	2.0	0.028 11.0

3.5.2. Analysis of feed/fodder samples for pesticides

Triplicate 1 µL of liquid sample was removed from bulk extracted sample and was placed into pre-weighed vial (3 µL). The vial was placed on autosampler (AOC-20i and AOC-20s, Perkin Elmer) and 1 µL of the sample was injected through GC. Data acquisition and reduction was achieved by using a Mass Spectrometer services data system or NIST software. The peak area of each pesticide on the GC trace was converted to mg L^{-1} and the concentration of each peak was calculated according to the formula given below.

Formula 1:

Concentration of analyte in the sample = Response factor x sample peak response

in µg/ml or mg/l { calculated from chromatogram}

Formula 2:

$$\text{Concentration of analyte in the sample} = \frac{\text{SamplePeakResponse (from chromatogram)}}{\text{Standard response factor}}$$

In µg/ml

Analytical Conditions of GCMS

Gas Chromatogram

Type of instrument	GC-MS Perkin Elmer Clara 600 series
Injection mode	Splitless
Inlet temperature	280°C
Glass Liner	Deactivated glass linear with glass wool (Restek)
Flow control mode	Constant linear velocity (47.2 cm sec^{-1})
He gas flow	1.3 ml per minute
Temperature programmed	80°C ramped to 280°C at 200°C per minute after a 30 sec delay
Oven temperature programmed	75°C for 3 minutes, then 25°C per minute ramp to 180°C, then by a 5°C per minute ramp to 300°C and held for 3 minutes and total hold time was 34.2 minutes.

Mass Spectrometer

Transfer line temperature	240°C
Ion source or trap temperature	230°C
Manifold temperature	120 °C
EI	10 µA filament current from 5 to 31 minutes to get 2.7 scans per sec
Acquisition mode	Scan
Mass Range	m/z 50, 550 m/z
Scan interval	0.3 sec
Injection volume	1 µL
Column	Capillary column Restek-Rxi-5 SIL MS, 30 m *0.25 mm *0.25 µm With 5% phenyl, 95% dimethyl polysiloxane.

3.5.3. Analysis of milk samples for heavy metals

The milk extract was analyzed through Atomic Absorption Spectrometer for heavy metals. Energy program of the furnace was optimized to obtain the best signal during atomization process. A blank solution of distilled water was used to check accuracy of the standard solutions and it was run after every 10 samples. The metal content was calculated using the formula

$$\text{Concentration} = \frac{\text{Concentration of the element through AAS(ppm) x sample volume}}{\text{Sample weight}}$$

mg L^{-1} or ppm

3.5.4 Analysis of milk samples for pesticides

Triplicate 1 µL of liquid sample was removed from bulk liquid sample and was placed into pre-weighed vial (3 µL). The vial was placed on autosampler (AOC-20i and AOC-20s, Perkin Elmer) and 1 µL of the sample was injected through GC. The peak area of

each pesticide on the GC trace was converted to mg L^{-1} and the concentration of each peak was calculated according to the formula given below.

Formula 1:

Concentration of analyte in the sample = Response factor x sample peak response

in µg/ml or mg/l { calculated from chromatogram}

Formula 2:

Concentration of analyte in the sample=SamplePeakResponse (from chromatogram)

In µg/ml Standard response factor

3.6. Calibration curve and standard preparation

3.6.1. Calibration curve and standard preparation of heavy metals

Calibration curve:

Calibration curves were drawn for lead, chromium, cadmium, cobalt, copper and nickel using linear regression. Each standard solution was measured 3 times and the mean was plotted. The range of linearity of the concentration versus absorbance curve is of great importance in determining the elemental concentration in milk samples. The standard stock solution of each element was prepared in concentrations of 2.5ppm, 5ppm, 10ppm from 1000mg/l stock solution according to the formula $C_1V_1=C_2V_2$. It was prepared for each element as follows:

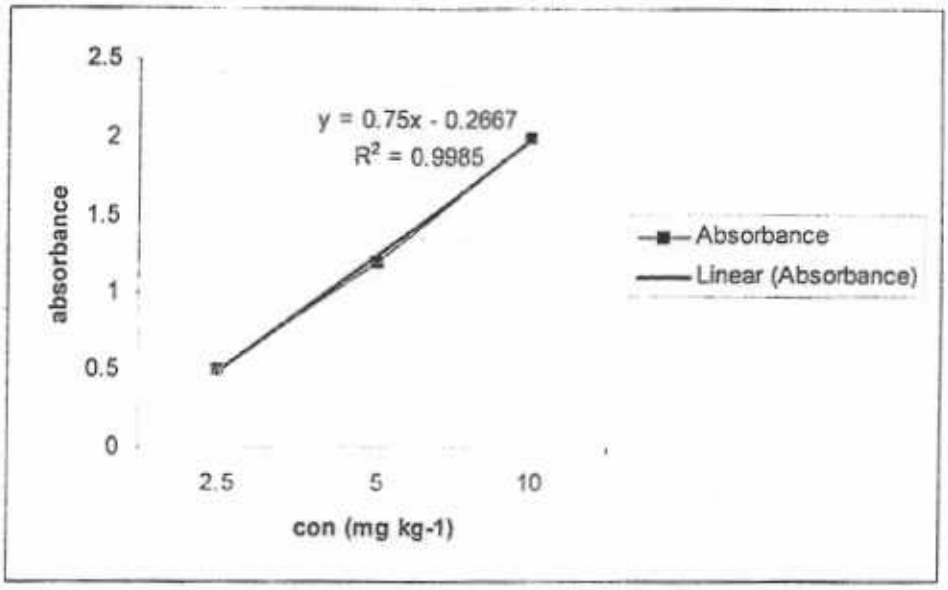


Figure 3.6.1. Calibration curve of standards vs absorbance .

Standard Preparation:

Lead, Nickel and Copper:

Dissolve 1.598gm of lead nitrate $Pb(NO_3)_2$ in 1% v/v HNO_3, 1 gm of Nickel metal and Copper metal in a minimum volume of 1+1 HNO_3 and dilute it to 1 L with 1% v/v HNO_3.

Cadmium and Cobalt:

Dissolve 1 gm of Cadmium metal and 1 gm of Cobalt metal in a minimum volume of 1+1 HCl and dilute it to 1 L with 1% v/v HCl

Chromium:

Dissolve 3.735gm of potassium chromate K_2CrO_4 in deionized water and dilute it to1L with deionized water.

3.6.2. Calibration curve and standard preparation of pesticides

The calibration curve is determined by the analysis of 5 calibration levels, i.e. 0.0005, 0.001, 0.01, 0.1 and 0.3 mg kg^{-1} (See Fig 3.6.2.1). Peak area was calculated and calibration curve was plotted for concentration vs response area or peak area. "The calibration curves were best fitted to a linear curve." The correlation coefficients (R) were 0.9947. The quantification was performed from the mean of calibration curves.

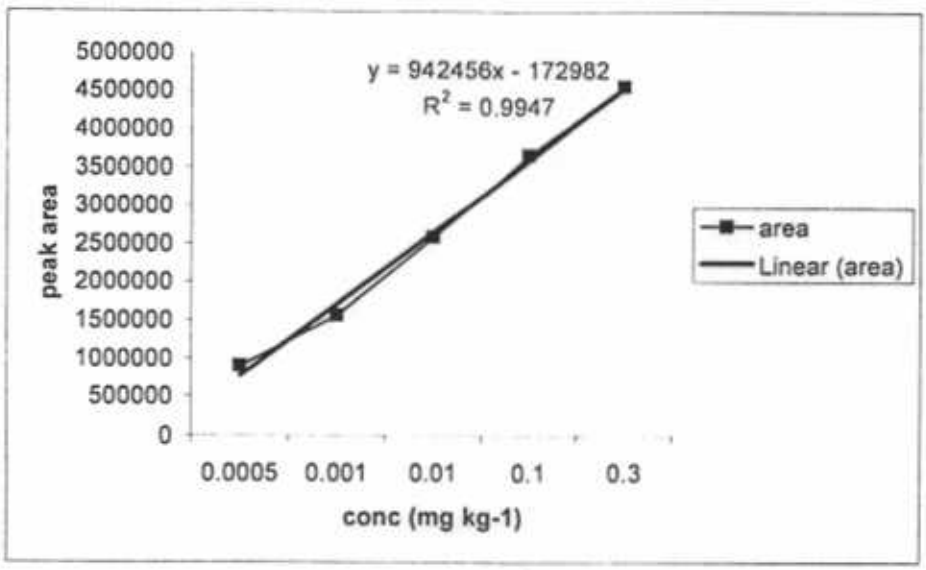


Figure 3.6.2.1. Calibration curve of cypermethrin at five different concentration levels ranged from 0.0005 to 0.3 mg kg^{-1}. Delete the labels area/Lnear (area) from graph.

Cypermethrin, Chlorpyrifos, Profenofos, Emamectin and Imidacloprid standards were purchased from Fluka (Germany). For solid/liquid standards an aliquot was prepared by adding 1 mg or 1 mL of standards to 9 mL of acetonitrile. The aliquot was shaken for a minute and allowed to settle for 5 minutes. Serial dilutions were prepared by taking 1 mL and diluting to 10 mL with acetonitrile up to 10^{-5}. Aliqout (2.5 µL) was removed from

each dilution and was placed in a glass vial measuring 3 µL. The vial was placed on autosampler and was analyzed with GCMS. The peak area of each standard on GC trace was converted to mg/ L^{-1} of the milk. The response factor of each external standard was calculated by taking the mean of response factor of 5 step dilutions calibration curve using following formulas.

Formula 1: Response Factor of Analyte Standard = $\frac{\text{Concentration of Standard}}{\text{Response or Peak area of Standard \{Calculated from chromatogram\}}}$

Formula 2: Response Factor of Standard = $\frac{\text{Standard peak response(from chromatogram)}}{\text{Standard concentration}}$

3.6.3. Quality assurance and quality control

For quality assurance and quality control of heavy metals in milk samples, a whole standard reference powder milk sample (NIST SRM 8435) and corn bran (NIST RM 8433) were purchased from U.S.A. The reference standards were used to compare the content of heavy metals in milk and diet samples of this study. Standard reference material for powder milk sample (NIST SRM 8435) and corn bran (NIST RM 8433) was used for precision and quality control was measured with and analyzed with Perkin Elmer 700 analyst. "The description of the analytical precision is defined as relative standard deviation (RSD) in percentage and is obtained using a formula: standard deviation/mean of sample. Accuracy was estimated through certified standard values in percentage recovery. To ensure the quality of results triplicate sub samples were analyzed and blank

and standard samples was also run for calibration of AAS. "Standard curve with liner regression and better relative standard deviations that can be employed to determine heavy metals content was calibrated." Periodic calibration of standards after 10 samples was performed in order to evaluate the accuracy, calibration and reliability of AAS. The accuracy was examined through quality control test in which standard reference measurement was compared with the sample measurement and the AA reading was used unless the recovery of elements in the sample was ± 15 at 15%, which is the lower limit for determination and standard deviation of triplicate samples less than 15% (Siddique, S.,2010)."

3.7. Statistical analysis

Statistical analysis was done by using Statistical Package for Social Sciences version 16. During analysis frequencies and correlation coefficient were taken out. The data was presented as frequency tables and graphs.

Chapter 4
RESULTS

4.0. RESULTS

4.1 Heavy metals

4.1.1. Calibration curve

See under section 3.6.1 in Chapter 3.0 Method and Materials

4.1.2. Method validation

For quality assurance and quality control of heavy metals in milk samples, a whole standard reference powder milk sample (NIST SRM 8435) and corn bran (NIST RM 8433) were purchased from U.S.A. The reference standards were used to compare the content of heavy metals in milk and diet samples of this study. Standard reference material for powder milk sample (NIST SRM 8435) and corn bran (NIST SRM 8433) was used forl precision and quality control and analyzed with Perkin Elmer 700 analyst. "The description of the analytical precision is defined as relative standard deviation (RSD) in percentage and is obtained using a formula: standard deviation/mean of sample. Accuracy was estimated through certified standard values in percentage recovery. To ensure the quality of results triplicate sub samples were analyzed and blank and standard samples was also run for calibration of AAS. Standard curve with liner regression and better relative standard deviations that can be employed to determine heavy metals content was calibrated. Periodic calibration of standards after 10 samples was performed in order to evaluate the accuracy, calibration and reliability of AAS. The accuracy was examined through quality control test in which standard reference measurement was compared with the sample measurement and the AA reading was used unless the recovery of elements in the sample was ± 15 at 15%, which is the lower limit for

determination and standard deviation of triplicate samples less than 15% (Samina, S., 2011)."

Table 4.1.2.1 differences in the observed and certified concentration (mg kg^{-1}) of trace elements in whole milk powder and animal feed standard reference materials (SRM 8435, 8433)

	Concentration mg kg^{-1} In SRM 8435		Concentration mg kg^{-1} In SRM 8433	
Element	Observed value	Certified	Observed value	Certified value
Cd	Nd	0.0002	NA	NA
Co	0.0018±0.001	0.003	NA	NA
Cr	0.47±0.006	0.5	NA	NA
Cu	0.432±0.007	0.46±0.23	NA	NA
Ni	0.008±0.003	0.01	NA	NA
Pb	0.103±0.003	0.11±0.05	NA	NA

4.1.3. Method detection limits

The instrument has been calibrated according to the guidelines of the manufacturer

Metal	Limit (ug/l)	Limit (ppm)
Cadmium	0.8	0.0008
Cobalt	9.0	0.009
Chromium	3.0	0.003
Copper	1.5	0.0015
Nickel	6.0	0.006
Lead	15	0.015

4.1.4. Mean elemental concentration of whole cow milk

Table 4.1.4.1 shows the difference in the mean content of heavy metals (Cd, Cr, Co, Cu, Ni and Pb) in dairy cattle's milk collected from urban and rural dairy farms within Peshawar district. There was significant difference ($p<0.05$) in the mean content of Cd, Cr, Co, Cu and Ni in the dairy cattle milk farmed in the urban and rural farms. Virtually there is a trend that low content of these elements was observed in the dairy cattle milk farmed at rural areas than urban areas. Nonetheless, there was no significant difference in the amount of Pb between urban and rural farms. Virtually, there was a trend that greater amount of Pb was found in the dairy milk of urban farm 1 compared to other four urban and rural farms (2 to 5). Exceptionally low level of Cr was noted in the dairy milk of rural farms (4&5) than urban farms (1 to 3). High level of Cr was noted in the dairy milk of urban farm (1) than any of the rural or urban farms (2 to 5). Cadmium shows significant difference between urban and rural farms. Urban farms (1 to 3) showed high content of Cd than rural farms (4&5) whereas the same was observed with Ni content in the dairy cattle milk between rural and urban farms. Like Cd and Ni, Co showed the same trend except that there were a significant difference ($p<0.05$) in its content between rural farms. Nonetheless, Co was significantly different in urban farms than rural farms. Virtually there was no significant difference in the content of Cd among urban farms (1 to 3) but was significantly greater in urban farms (1 to 3) than rural farms. There was no significant difference in the levels of any of the heavy metals between rural farms 4 and 5 except for Co. All heavy metals were found to be significantly greater in one of the urban farm (1) than other four rural and urban farms (2 to 5).

Table 4.1.4.1. Heavy metals mean concentration (mg L^{-1}) in dairy milk collected from rural and urban dairy farms located within Peshawar district

Farm No	Location	Cd	Co	Cr	Cu	Ni	Pb
1	Urban	0.70b	0.79c	0.51c	1.67c	0.91c	2.45
2	Urban	0.60b	0.60c	0.09b	1.22b	0.83c	2.12
3	Urban	0.50b	0.30b	0.03b	1.29b	0.63b	2.35
4	Rural	0.043a	0.13a	0.007a	0.08a	0.024a	1.96
5	Rural	0.035a	0.29b	0.009a	0.09a	0.024a	2.30

*means (n=3) showed by different letters within columns are significantly different at the 5% level of probability.

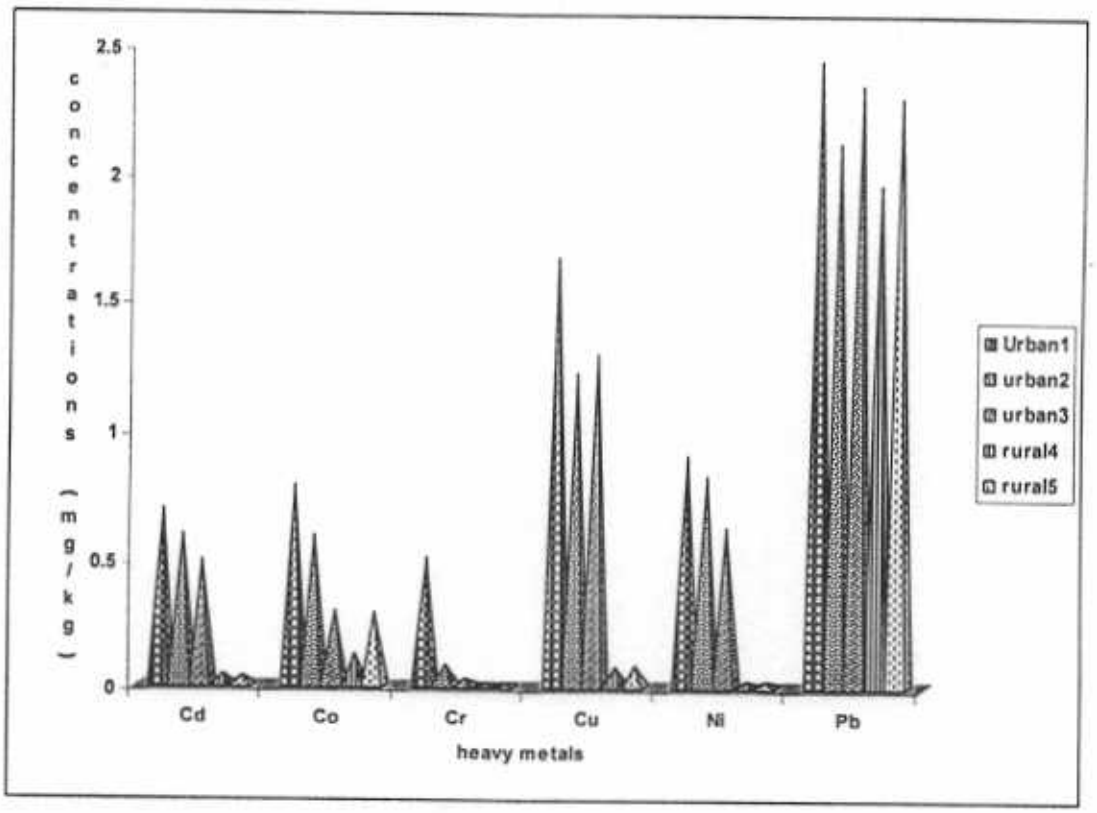


Figure 4.1.4.1. Heavy metals mean concentration (mg L^{-1}) in dairy milk collected from rural and urban dairy farms located within Peshawar district

Table 4.1.4.2 shows the difference in the mean content of heavy metals (Cd, Cr, Co, Cu, Ni and Pb) in pasteurized and dry milk commercially available in Peshawar. Lead content remained same in pasteurized and dry milk whereas Cd content was significantly greater ($p<0.05$) in Nestle, Tarang and Everyday milk samples than other pasteurized and dry milk samples. Chromium was found to be low in Nestle and dry milk than Tarang, Haleeb and Olper. Cobalt was significantly greater ($p<0.05$) in Nestle+ Haleeb and Nido+Milac than other pasteurized and dry milk samples. Copper content was significantly greater ($p<0.05$) in Haleeb and Milac milk samples than other pasteurized and dry milk samples. Nickel content was low in Tarang followed by Haleeb, Nido, and Olper and was greater in Nestle and Everyday milk samples.

Table 4.1.4.2. Heavy metals mean concentration in (mg L^{-1}) in pasteurized and dry milk

Pasteurized milk	Cd	Cr	Co	Cu	Ni	Pb
Haleeb	0.64a	0.017b	1.30c	1.90c	0.49b	0.012
Nestle	0.93b	0.0013a	0.84b	0.66a	0.73c	0.012
Tarang	1.06b	0.017b	0.66a	1.04b	0.004a	0.012
Olper	0.52a	0.023b	0.55a	0.65a	0.45b	0.012
Dry powder milk						
Nido	0.60a	0.001a	1.12c	0.88a	0.20b	0.012
Everyday	1.23b	0.001a	0.44a	1.13b	0.99c	0.011
Milac	0.52a	0.001a	0.98b	1.45c	0.18d	0.011
Good milk	0.96	0.001	1.01	1.05	0.67	0.014

*means (n=3) showed by different letters within columns are significantly different at the 5% level of probability.

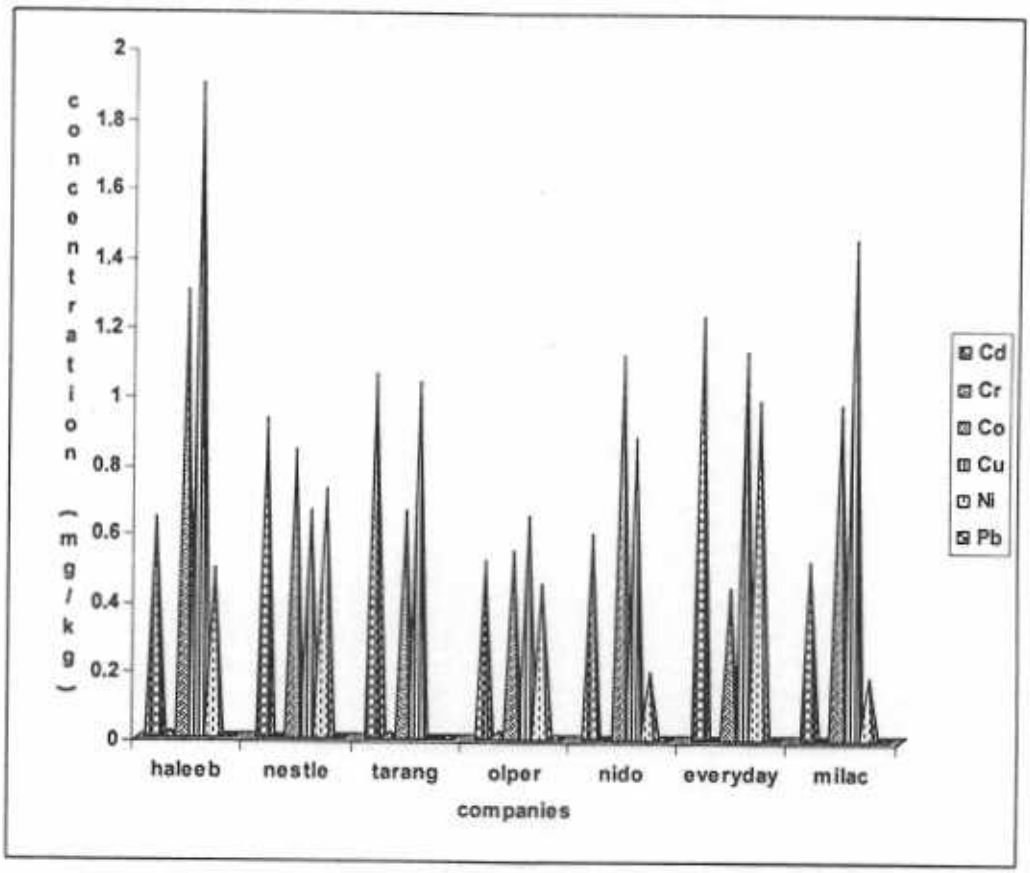


Figure 4.1.4.2. Heavy metals mean concentration (mg L^{-1}) in pasteurized and dry milk

Tables 4.1.4.3a to 3c show the descriptive statistic of heavy metals in raw, pasteurized and dry milks. There was great variation between minimum and maximum content of all heavy metals in fresh milk whereas no variation was noted between the mean and median of Cu and Cd whereas there was a greater difference in the mean and median of Cr, Co, Ni and Pb. In contrast to that minimum and maximum contents of Cr and Pb in pasteurized and dry milk remained same whereas great variation was noted among means of other four elements. Mean and median of both milk remained same with little variation between them for any of the elements. The mean values of Ni in both the categories were twice that of median. Kurtosis and Skewness characterize the location and variability of

data set. Skewness measures the symmetry of the data set whereas kurtosis measures the peak or flattness of data set with normal distribution. The kurtosis and skewness of Co and Ni shows their normal distribution in fresh milk. Platykurtic and peaky distribution of Cu, Pb+ Cd and Cr was noted in milk samples (Table 3.3a). Normal distribution of Cu and Pb was noted in pasteurized milk because kurtosis is up to 3 whereas platykurtic and peaky distribution was noted for other trace elements in pasteurized milk. Copper was normally and Ni was peaky distributed in dry milk whereas for all other trace elements platykurtic distribution was observed in dry milk samples (Tables 4.1.4.3b&c).

Tables 4.1.4.3a. Descriptive statistic of heavy metals concentration in raw dairy milk collected from urban and rural dairy farms.

Elements (mg L^{-1})	Minimum	Maximum	Mean	Median	SE	Kurtosis	Skewness
Cd	0.0006	1.15	0.51	0.45	0.042	-0.890	0.323
Cr	0.001	0.8	0.038	0.0025	0.016	39.4	6.06
Co	0.007	2.6	0.41	0.007	0.09	3.15	1.83
Cu	0.14	3.2	1.25	1.12	0.09	0.19	0.78
Ni	0.004	3.2	0.54	0.027	0.10	2.08	1.51
Pb	0.012	11	2.2	0.012	0.46	0.11	1.18

Tables 4.1.4.3b. Descriptive statistic of heavy metals concentration in pasteurized milk

Elements ($mg\ L^{-1}$)	Minimum	Maximum	Mean	Median	SE	Kurtosis	Skewness
Cd	0.25	1.59	0.79	0.8	0.13	-0.83	0.51
Cr	0.001	0.05	0.015	0.0015	0.0063	-0.96	0.99
Co	0.006	2.1	0.74	0.66	0.21	-0.77	0.62
Cu	0.34	2.8	1.06	0.89	0.19	2.8	1.49
Ni	0.004	1.45	0.42	0.28	0.13	0.37	0.95
Pb	0.011	0.012	0.011	0.012	0.0001	2.64	-0.205

Tables 4.1.4.3c. Descriptive statistic of heavy metals concentration in dry milk commercially available in Peshawar.

Elements ($mg\ L^{-1}$)	Minimum	Maximum	Mean	Median	SE	kurtosis	Skewness
Cd	0.24	1.64	0.86	0.755	0.16	-1.14	0.54
Cr	0.001	0.001	0.001	0.001	0	-2.57	-1.12
Co	0.007	2.1	0.76	0.75	0.24	-1.005	0.52
Cu	0.53	2.8	1.18	0.89	0.21	2.9	1.62
Ni	0.001	1.68	0.47	0.20	0.19	0.18	1.13
Pb	0.012	0.012	0.012	0.012	$6.94*10^{-11}$	-2.57	1.18

Table 4.1.4.4 shows the difference in the heavy metals concentration among raw, pasteurized and dry milk. There was no significant difference in the mean content of Cr, Cu, Ni and Pb between pasteurized and dry milk whereas all of them were higher in fresh milk as compared to pasteurized and dry milk. Concentrations of lead was significantly greater ($p<0.05$) in fresh dairy milk than pasteurized milk and dry milk. Contrary to that low content of Cd and Co was noted in fresh dairy milk than pasteurized and dry milk. All the observed concentrations are higher than the standard permissible limits except for lead and chromium in pasteurized and dry milk.

Table 4.1.4.4. Heavy metals mean concentration in fresh, pasteurized and dry milk.

Types of Milk	Cd	Cr	Co	Cu	Ni	Pb
Fresh dairy milk	0.52a	0.43c	0.13a	1.27	0.63	2.23b
Pasteurized dairy milk	0.78b	0.014b	0.84b	1.06	0.42	0.012a
Commercially available dry milk	0.78b	0.001a	0.84b	1.15	0.46	0.012a

*means (n=3) showed by different letters within columns are significantly different at the 5% level of probability

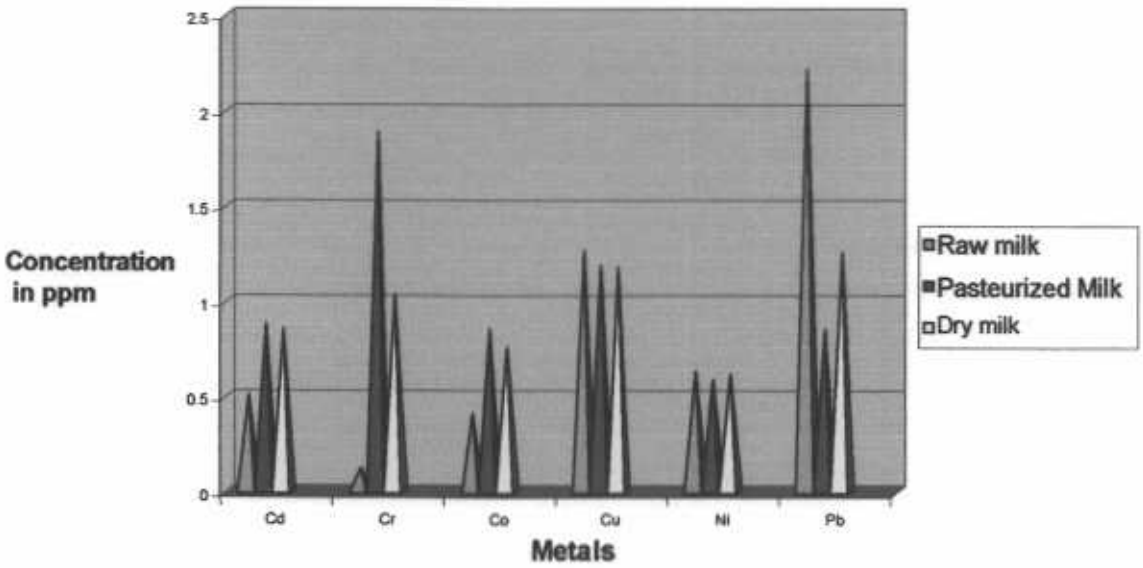


Figure 4.1.4.4. Comparison of heavy metals in fresh, pasteurized and dry milk samples

Tables 4.1.4.5a to f show the differences in the mean content (mg kg^{-1}) of heavy metals in the diet available locally given to dairy cattle farmed in the urban and rural dairy farms within Peshawar district. Table 4.1.4.5a represents that there was no significant difference in the mean value of Cd, Cr, Cu, Ni and Pb in any of the farm except for Co. Cobalt was found to be significantly different in urban farm 3 and rural farms than other urban farms. Nonetheless, there was significantly difference in Co content between rural farms 1 and 2, whereas high content of Co was noted in urban farm 3 than other two urban and rural farms. The low content of Co was noted in rural farm 4 than other farms.

Table4.1.4.5a. Heavy metals mean concentration in cotton khal diet given to dairy cattle farmed at urban and rural farms

Farm No	Location	Cd	Cr	Co	Cu	Ni	Pb
1	Urban	0.32	4.8	0.60b	80.92	2.12	3.8
2	Urban	0.39	5.0	0.42a	85.24	1.93	4.2
3	Urban	0.30	4.7	0.97c	83.56	2.84	4.6
4	Rural	0.38	5.4	0.34a	84.20	2.65	3.5
5	Rural	0.36	5.2	0.81c	82.67	2.01	4.2

*means (n=3) showed by different letters within columns are significantly different at the 5% level of probability

Table 4.1.4.5b shows the difference in the mean value of heavy metals in sugarcane khal diet given to dairy cattle farmed at urban and rural farms located within Peshawar district. The table represents that Cd was completely absent in all of the sugarcane khal diet irrespective of the farms. There was no significant difference in the mean value of all trace elements except for Cu in any of the farms. Copper was found to be low in urban farm 2 than other rural and urban farms. Nevertheless greater content of Cu was noted in the sugarcane khal diet given to the dairy cattle farmed at rural dairy farms whereas low content of Cu was observed in the diet of dairy cattle farmed at urban farms than rural farms.

Table 4.1.4.5b. Heavy metals mean concentration in sugarcane khal diet given to dairy cattle farmed at urban and rural farms

Farm No	Location	Cd	Cr	Co	Cu	Ni	Pb
1	Urban	nd	19.79	0.02	4.82b	1.12	2.91
2	Urban	nd	20.83	0.08	3.68a	1.19	2.87
3	Urban	nd	22.52	0.06	5.92b	1.17	3.12
4	Rural	nd	21.84	0.01	6.31c	1.04	3.48
5	Rural	nd	21.96	0.01	6.27c	2.01	2.84

*means (n=3) showed by different letters within columns are significantly different at the 5% level of probability

Table 4.1.4.5c shows the difference in the mean value of heavy metals in wheat bran diet given to dairy cattle farmed at urban and rural farms located within Peshawar district. There was no significant difference in the mean value of Cd, Cr and Co in wheat bran diet given to dairy cattle farmed at rural and urban farms. However there was a trend that greater content of Cr was noted in the wheat bran given to dairy cattle farmed at rural farms than urban farms. Whereas, the content of Cd, Cr and Co in wheat bran diet remained more or less same in both urban and rural farms. Nevertheless, high mean value of Cu, Ni and Pb was noted in the wheat bran given to dairy cattle farmed at rural farms than urban farms. Nevertheless there was significantly low ($p<0.05$) content of Cu, Ni and Pb was observed in wheat bran given to dairy cattle farmed at urban farm 1 than urban farms 2 &3 and rural farms. Lead, Cu and Ni content was significantly greater ($p<0.05$) in rural farms than urban farms.

Table 4.1.4.5c. Heavy metals mean concentration in wheat bran diet given to dairy cattle farmed at urban and rural farms

Farm No	Location	Cd	Cr	Co	Cu	Ni	Pb
1	Urban	0.10	1.92	0.06	0.22a	0.21a	4.28a
2	Urban	0.04	1.42	0.01	0.37b	0.62b	5.67b
3	Urban	0.15	1.80	0.08	0.42b	0.91b	5.23b
4	Rural	0.08	2.12	0.02	0.58c	1.73c	6.94c
5	Rural	0.09	2.01	0.01	0.65c	1.36c	6.48c

*means (n=3) showed by different letters within columns are significantly different at the 5% level of probability

Table 4.1.4.5d shows the difference in the mean value of heavy metals in wheat grain diet given to dairy cattle farmed at urban and rural farms located within Peshawar district. There was no significant difference in the mean value of Cd, Cr and Ni in wheat grain diet given to dairy cattle farmed at rural and urban farms. However there is a trend that greater content of Cr was noted in the wheat grain given to dairy cattle farmed at rural farms than urban farms. Whereas, the low content of Cd was observed in the wheat grain diet given to dairy cattle farmed at rural farm 4 than rural farm 5 and all other urban farms. There was no significant difference in the content of Co in the wheat grain diet given to dairy cattle farmed at rural and urban farms. Nonetheless greater content of Co was noted in wheat grain diet of urban farm 3. Contrary to that there is trend that greater content of Ni was noted in the wheat grain diet given to dairy cattle in all farms except for rural farm 5. Unlike Cd, Cr, Co and Ni, Cu + Pb showed different trend in their content in wheat grain diet between rural and urban farms. The high content of Cu and Pb was found in the wheat grain diet given to dairy cattle farmed at rural farms than urban farms. Virtually there is no significant difference in the mean value of Cd, Cr and Co in the wheat grain diet given to the dairy cattle at both farms whereas, Cu, Ni and Pb mean content varied in the wheat diet between farms.

Table 4.1.4.5d. Heavy metals mean concentration in wheat grain diet given to dairy cattle farmed at urban and rural farms

Farm No	Location	Cd	Cr	Co	Cu	Ni	Pb
1	Urban	0.13	8.14	0.03a	0.18a	6.30	5.10a
2	Urban	0.14	8.14	0.03a	0.19a	6.21	4.64a
3	Urban	0.16	8.20	0.09b	0.23a	6.30	5.14a
4	Rural	0.09	8.64	0.01a	0.34b	6.84	5.67b
5	Rural	0.15	8.81	0.02a	0.32b	5.83	6.48b

*means (n=3) showed by different letters within columns are significantly different at the 5% level of probability

Table 4.1.4.5e shows the difference in the mean value of heavy metals in the maize diet given to dairy cattle farmed at urban and rural farms located within Peshawar district. There was no significant difference in the mean value of Cd, Cr and Co in maize diet given to dairy cattle farmed at rural and urban farms. However there is a trend that low content of Co was found in maize diet given to dairy cattle farmed at urban farm 1 and high content was observed in the diet of dairy cattle farmed at rural farm 4. There is no clear trend in the mean value of Cu, Ni and Pb was observed in the maize diet given to dairy cattle farmed at rural and urban farms. However, Ni was found to be greater in the maize diet of urban and rural farms (3 to 5) than urban farms (1&2). Lead wa found to be greater and same in all rural and urban farms than rural farm 4. Nonetheless, low content of Cu was found in the maize diet given to dairy cattle farmed at rural farm 5 and urban farm (1&2) than other rural and urban farms (3&4). Contrary to that greater content of Cu was noted in the maize diet of rural farm 4 and urban farm 3 than other rural and urban farms.

Table 4.1.4.5e. Heavy metals mean concentration in maize diet given to dairy cattle farmed at urban and rural farms

Farm No	Location	Cd	Cr	Co	Cu	Ni	Pb
1	Urban	0.22	22.1	0.09	0.18a	0.92a	7.97b
2	Urban	0.20	24.4	0.16	0.19a	1.08b	8.02b
3	Urban	0.28	27.0	0.15	0.28b	1.95c	8.48b
4	Rural	0.21	26.3	0.19	0.20b	2.3c	7.63a
5	Rural	0.29	23.5	0.14	0.10a	2.4c	8.23b

*means (n=3) showed by different letters within columns are significantly different at the 5% level of probability

Table 4.1.4.5f shows the difference in the mean value of heavy metals in mixed diet (mixture of cotton khal, sugar cane khal, shotal, maize plant) given to dairy cattle farmed at urban and rural farms located within Peshawar district. There was no significant difference in the mean value of Cd, Cr, Cu and Pb in mixed diet given to dairy cattle farmed at rural and urban farms. However there is a trend that low content of Cd and Cu was found in mixed diet given to dairy cattle farmed at urban farm 1 than all other farms whereas high content of these elements was observed in mixed diet given to dairy cattle farmed at urban farms (2&3) and rural farm (4). Unlike Cd, Cr, Cu and Pb, Ni was significantly different ($p<0.05$) in urban farm 2 than 1&3. Unlike Ni, Co was found to be significantly greater ($p<0.05$) in mixed diet given to dairy cattle farmed at rural farm 4 than other urban and rural farms. None of the trace elements were detected in rural farm 5 whereas Pb and Ni were completely absent in rural farms (4&5).

Table 4.1.4.5f. Heavy metals mean concentration in mixed diet given to dairy cattle farmed at urban and rural farms

Farm No	Location	Cd	Cr	Co	Cu	Ni	Pb
1	Urban	0.25	4.3	0.21a	0.28	0.86a	4.12
2	Urban	0.30	3.9	0.34b	0.39	1.90b	4.48
3	Urban	0.29	3.2	0.42b	0.40	0.92a	3.87
4	Rural	0.33	4.1	0.65c	0.31	Nd	Nd
5	Rural	Nd	Nd	Nd	Nd	Nd	Nd

*means (n=3) showed by different letters within columns are significantly different at the 5% level of probability

Table 4.1.4.6 shows the difference and similarities in the overall mean content of heavy metals (Cd, Co, Cr, Cu, Ni and Pb) in various forms of diet available locally irrespective of farms. Cadmium was not detectable in sugarcane khal and was greater in cotton khal, maize and mixed diets whereas low content was noted in other diets. Low content of all trace elements except for Co was noted in wheat bran diet, whereas exceptionally high content of Cr and Cu was noted in maize and cotton khal diets. Virtually cotton khal was found to be significantly greater ($p<0.05$) in Cu than other diet whereas low Cu was observed in wheat bran followed by wheat grain and maize diet. Chromium was greater in sugarcane khal and maize diet followed by wheat grain and low content was noted in wheat bran and cotton khal diets. Like Cr, Pb was greater in maize diet than all other diets and was low in wheat bran than other diets. Cobalt was found to be greater in wheat bran followed by cotton khal and mixed diet and low content was observed in sugarcane khal and wheat grain than other diets. Nickel was significantly different in wheat grain followed by cotton khal and then sugarcane khal, maize and mixed diet, whereas low content of Ni was found in wheat bran.

Table 4.1.4.6. Heavy metals mean concentration in various diets given to dairy cattle farmed at urban and rural farms.

Types of diet	Cd	Cr	Co	Cu	Ni	Pb
Cotton khal	0.35b	5.02b	0.63c	83.3d	2.31c	4.06b
Sugar cane khal	nd	21.34d	0.03a	5.4c	1.30b	3.04b
Wheat bran	0.09a	0.09a	1.85d	0.03a	0.45a	0.96a
Wheat	0.13a	8.40c	0.04a	0.27b	6.56d	5.7b
Maize	0.24b	24.66d	0.15b	0.19b	1.73b	8.06c
Mixed	0.27b	3.84b	0.43c	0.34c	1.68b	4.11b

*means (n=3) showed by different letters within columns are significantly different at the 5% level of probability.

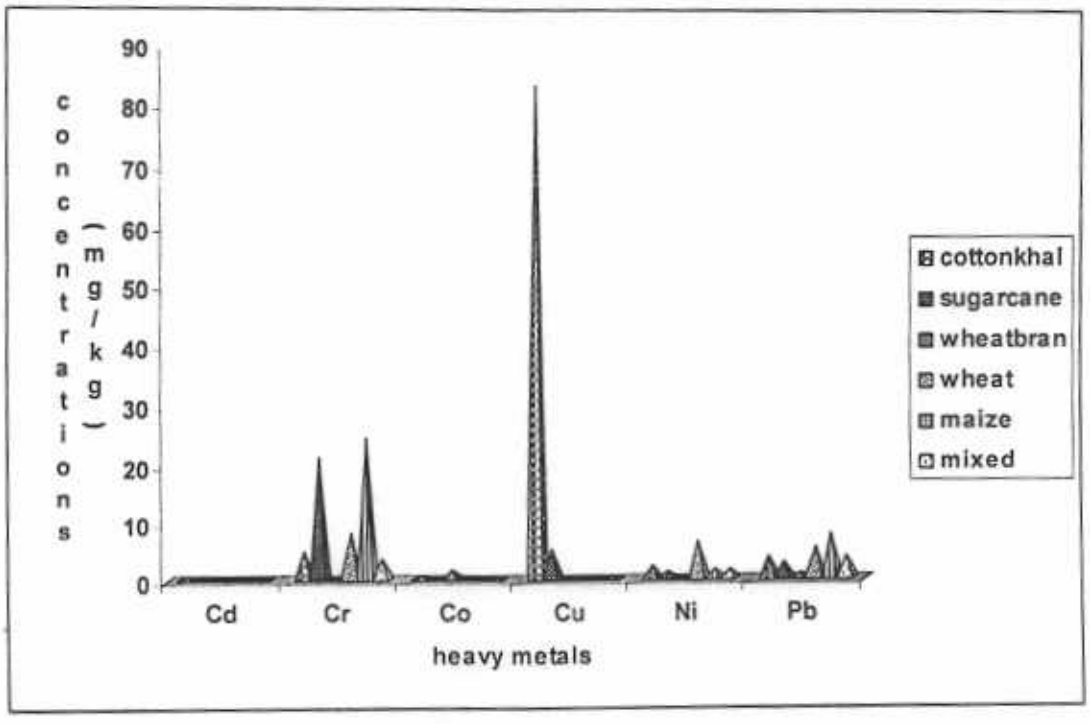


Figure 4.1.4.6. Comparison between heavy metals content of all diets given to cattle.

Table 4.1.4.7 shows the descriptive statistic of heavy metals in various cattle diets. There was great variation between minimum and maximum content of metals in various cattle diets. Mean and median was more or less same except for Cu. Kurtosis shows normal distribution for Co whereas peaky and platykurtic distribution was observed for all other trace elements in various cattle diets.

Table 4.1.4.7. Descriptive statistics of heavy metals in cattle diet

Elements (mg kg^{-1})	Minimum	Maximum	Mean	Median	SE	kurtosis	Skewness
Cd	0.04	0.39	0.24	0.28	0.02	-0.84	-0.50
Cr	1.42	27.0	11.0	8.14	1.64	-1.3	0.64
Co	0.01	0.97	0.20	0.085	0.046	2.14	1.67
Cu	0.1	85.2	14.99	0.83	5.69	1.63	1.87
Ni	0.21	7.62	2.4	1.9	0.39	0.33	1.30
Pb	2.84	8.48	5.12	4.26	0.32	-0.98	0.50

Figure 4.1.4.7. Comparison of heavy metals content between cattle diet and milk.

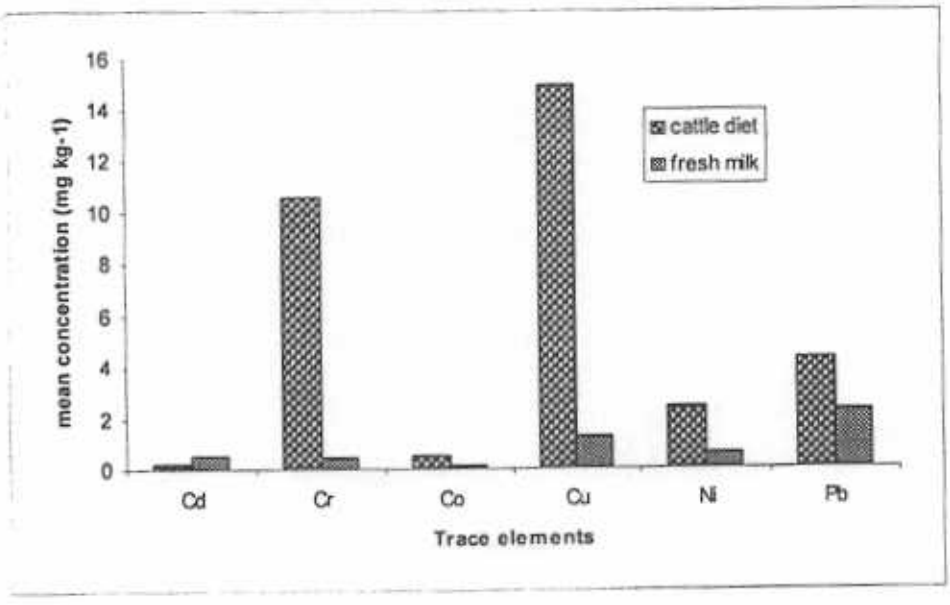


Table 4.1.4.8. Correlation between heavy metal content of dairy milk and animal feed.

Cadmium	Pearson's R	T Value	P value
Mixed feed	0.136	0.955	0.345
Maize	0.134	0.935	0.355
Wheat grain	0.012	0.08	0.936
Wheat bran	0.173	1.217	0.230
Sugarcane khal	0.163	1.145	0.258
Cotton khal	0.093	0.648	0.520
Chromium			
Mixed feed	0.215	1.522	0.135
Maize	0.133	0.929	0.357
Wheat grain	0.017	0.117	0.908
Wheat bran	0.056	0.391	0.698
Sugarcane khal	0.114	0.795	0.430
Cotton khal	0.192	1.353	0.182
Cobalt			
Mixed feed	0.036	0.249	0.804
Maize	0.027	0.190	0.850
Wheat grain	0.075	0.524	0.603
Wheat bran	0.087	0.605	0.548
Sugarcane khal	0.163	1.145	0.258
Cotton khal	0.101	0.702	0.486
Copper			
Mixed feed	0.030	0.207	0.837
Maize	0.014	0.099	0.921
Wheat grain	0.341	2.509	0.016
Wheat bran	0.269	1.934	0.059
Sugarcane khal	0.451	3.496	0.01
Cotton khal	0.134	0.935	0.355
Lead			
Mixed feed	0.260	1.868	0.068
Maize	0.153	1.076	0.287
Wheat grain	0.002	0.014	0.989
Wheat bran	0.023	0.159	0.874
Sugarcane khal	0.083	0.575	0.568
Cotton khal	0.179	1.260	0.214
Nickel			
Mixed feed	0.099	0.692	0.493
Maize	0.342	2.519	0.015
Wheat grain	0.054	0.373	0.711
Wheat bran	0.289	2.089	0.042
Sugarcane khal	0.092	0.643	0.523
Cotton khal	0.272	1.961	0.056

Figure 4.1.4.8. Correlation between Cd in raw milk & wheat bran

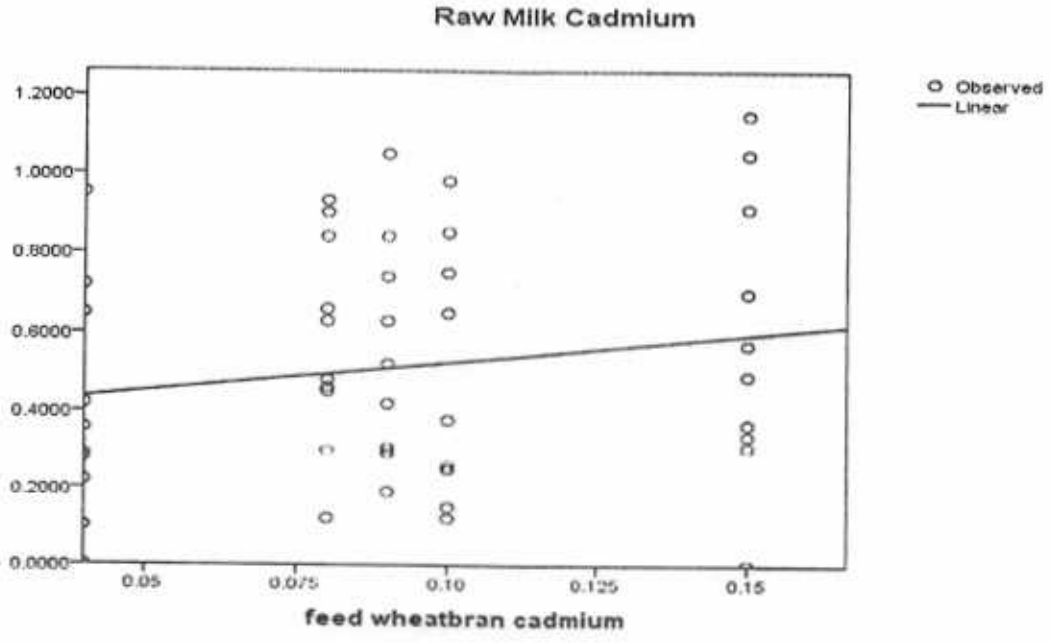


Figure 4.1.4.9. Correlation between Cd in raw milk & mixed feed

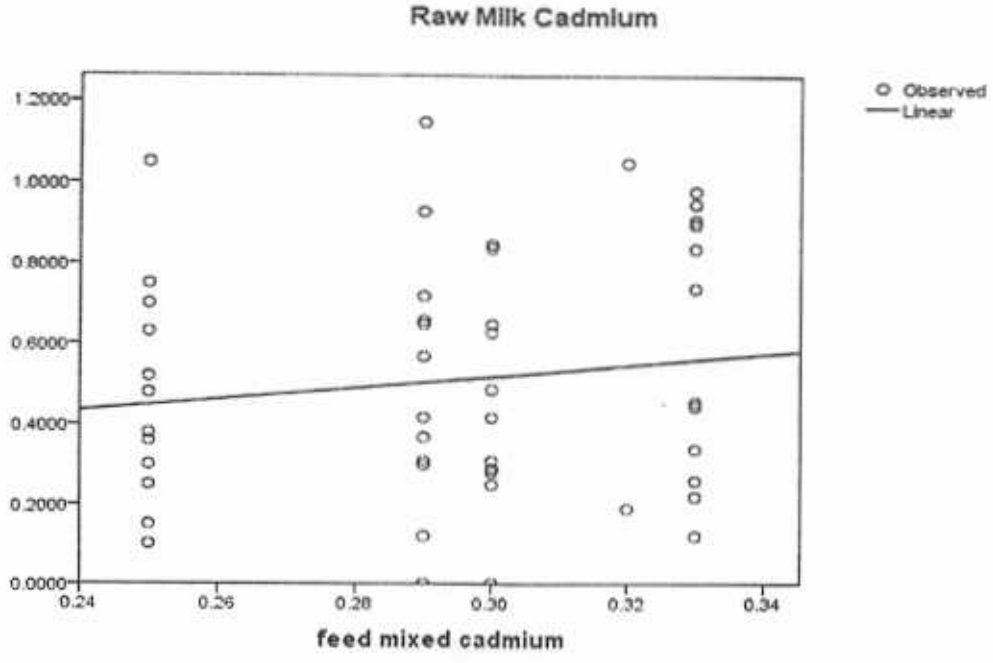


Figure 4.1.4.10. Correlation between Cd in raw milk & maize feed

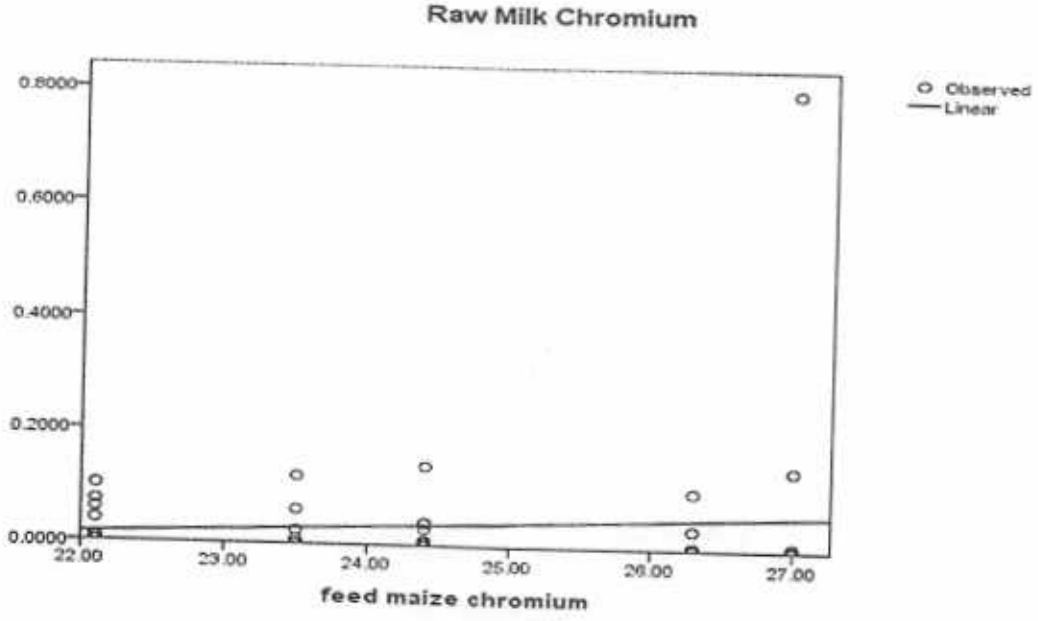


Figure 4.1.4.11. Correlation between Cr in raw milk & mixed feed

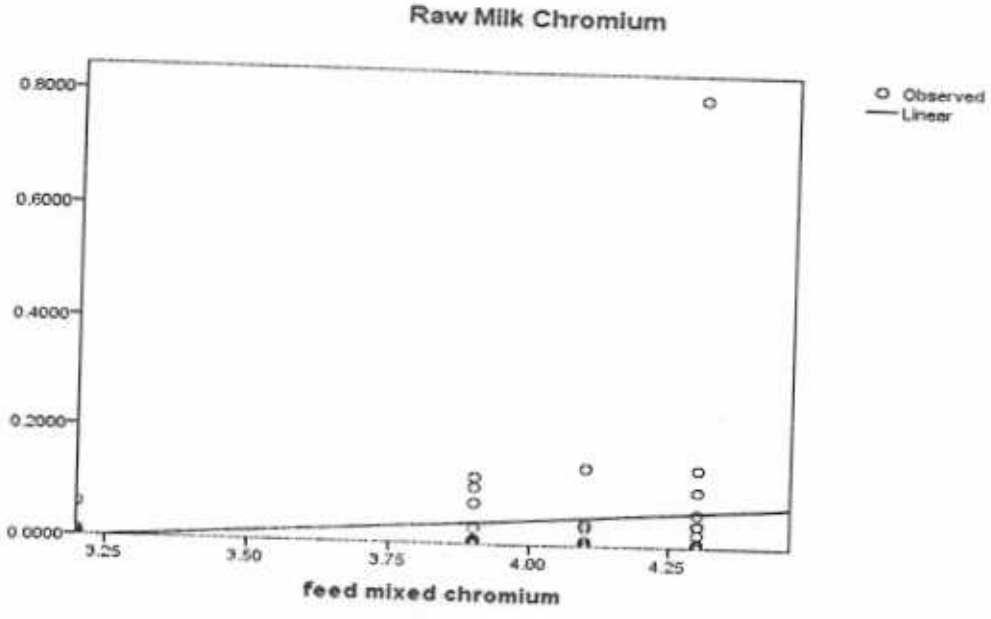


Figure 4.1.4.12. Correlation between Pb in raw milk & cotton khal

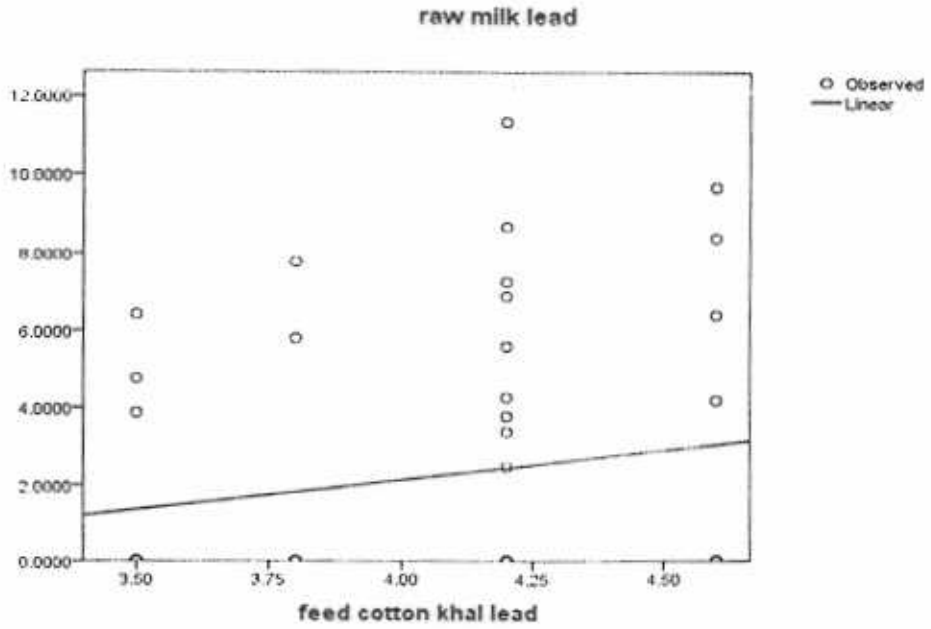


Figure 4.1.4.13. Correlation between Pb in raw milk & maize feed

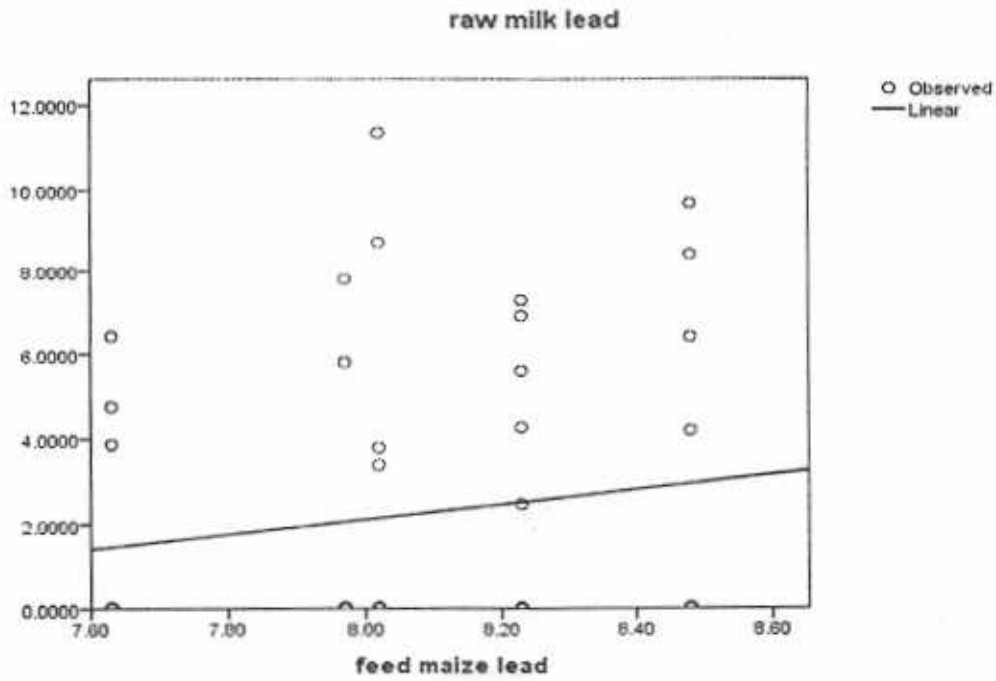


Figure 4.1.4.14 shows the recovery rate of heavy metals in different types of milk. The recovery rates for Cd, Cr, Co, Cu, Ni and and Pb in case of raw milk samples were 88%, 36%,40%, 100%, 52% and 48%, in case of pasteurized milk these were 90%, 64%, 51%,100%, 67% and 30% whereas in case of dry milk the recovery rates were 90%, 60%,65%, 100%, 70% and 25%.

Figure 4.1.4.14. Recovery rates of heavy metals in milk.

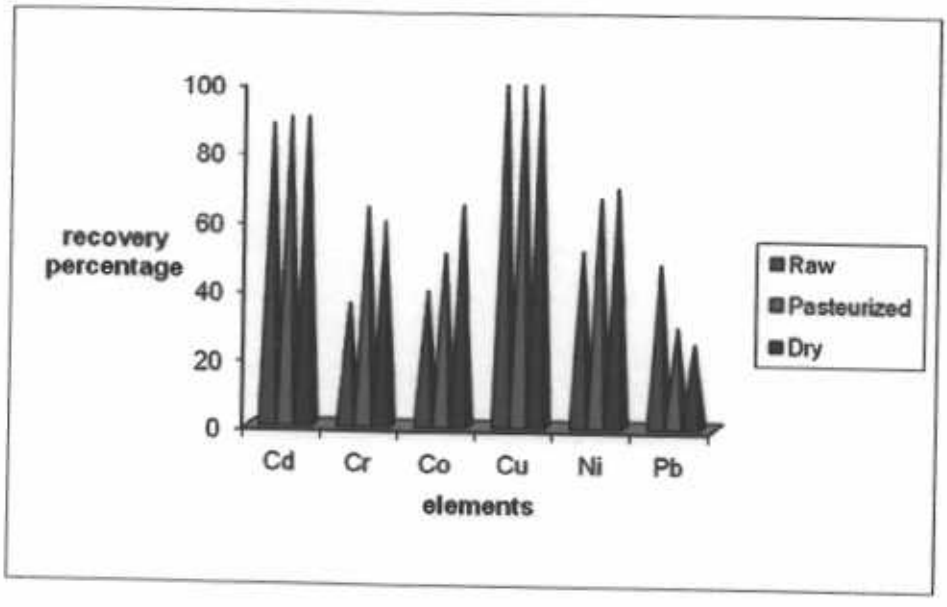


Table 4.1.5 shows the comparison of the heavy metal content in raw milk of this study with the world reported values for same elements in raw milk. Cadmium and Ni content ranged 0.52 and 0.63 mg kg^{-1} of raw milk in the present study which is greater than the Cd and Ni content reported for raw milk by other scientist wheras Cr content was greater in the present study than other studies except for the Cr content reported by Aslam et al (2011). Cobalt content in raw milk of this study was greater than the content reported by Enb et al (2009) and remained within the reported value for Co by Onianwa (1999). Copper content in the raw milk of present study remained within the reported value of Elsayed et al (2011) and Tripath et al (2009) whereas it was greater than other reported value for Cu. Pb contentof this study was lesser than that reported by Aslam et al (2011), Lante et al (2004) and Tajkarimi et al (2008) while it was more than that reported by others.

4.1.5. Comparison of metal content in milk (mg kg^{-1}) of the present study with the World reported data

Elements	This study	Aslam et al 2011	Caggiao et al 2005	Elsayed et al.2011	Enb et al 2009	Lante et al. 2004	Tripath et al 2009	Jan et al 2011	Tajkarimi et al.2008	Onianwa 1999
Cd	0.52	0.08-0.14	0.06	0.004 - 0.018	0.118	0.40	0.0007 - 0.001	NA	ND	.004-.009
Cr	0.43	0.98-1.27	ND	0.123-0.350	0.042	0.001	ND	0.0084	ND	0.005-0.030
Co	0.13	ND	ND	ND	0.008	ND	ND	NA	ND	0.03-0.12
Cu	1.27	ND	ND	0.614 - 1.23	0.212	0.0518	0.043 - 0.195	0.0148	ND	0.07-0.67
Ni	0.63	ND	ND	NA	0.006	ND	ND	0.0003	ND	0.04-0.09
Pb	2.23	41.76-43.41	0.2	0.135 - 0.614	0.084	5.23	0.017 - 0.0335	0.0132	7.9	0.03-0.18

Table 4.1.6 shows the comparison of heavy metals content in raw milk of this study with the maximum residue limits for same elements in raw milk. Except for Cu the content of all other elements in raw milk remained greater than the maximum residue limit for same elements.

4.1.6. Comparison of metal content in milk (mg kg^{-1}) of the present study with the maximum residue limits (MRLs)

Elements	This study	Max.residue limits (AOAC, 1989)	Max.residue limit Egypt (Abdallah, 2009)	Max.residue limit(IDF 1977,JECFA 1989; ATSDR, 1989)	Max. residue limitRomania (Florea et al., 2006)	Max.residue limit Czech (Hosnedlova et al., 2005)
Cd	0.52	<0.01	0.05	0.006 or 057-.071	0.05	NA
Cr	0.43	0.018	0.012	0.018	NA	NA
Co	0.13	NA	NA	0.009	NA	NA
Cu	1.27	3.65	0.4	0.01	NA	0.4
Ni	0.63	NA	NA	0.01	NA	NA
Pb	2.23	NA	0.3	0.025	0.02	NA

4.2. Pesticides

4.2.1. Calibration curve

See under section 3.6.2 in chapter 3.0 Method and Materials

4.2.2. Method validation

Precision-Repeatability

Precision of the analyte was measured from the standard deviation as described by Horwitz (1982). Precision of the method was estimated from the results of each analyte by an external standardization method with a standard deviation of less than 10%. Four replicates of milk and feed/fodder samples were spiked with four different levels of cypermethrin ranged from 0.001 to 0.1 mg per kg with standard deviation of ± 2 to 6. Repeatability in this validation was measured from the mean of 4-replicate of each sample (milk and diet). Repeatability was calculated as given ISO 5725-2. "The relative standard deviation of the repeatability must be less than or equal to the standard deviation proposed by Horwitz (1982)."

Accuracy –Recovery

The accuracy was determined from the mean of the recovery of standard samples at five concentration levels i.e. 0.0005, 0.001, 0.01, 0.1 and 0.3 mg kg^{-1} which ranged between 50% -100% The recovery rate of various pesticides ranged between 52.67% - 78.12%.

4.2.3. Method detection limit

Profenofos	0.0005 mg L^{-1}
Cypermethrin	0.01 mg L^{-1}
Chlorpyrifos	0.01 mg L^{-1}
Imidachloprid	-

"Quantification limits (LOQ) are calculated from the results at the lowest accepted standard level, as 6 times the standard deviation (absolute recover)."

Cypermethrin

Formula 1 (Quantitative Instrumental Analysis, 1998)

$$\text{Response Factor of Cypermethrin Standard} = \frac{\text{Concentration of Standard}}{\text{Response or Peak area of Standard \{Calculated from chromatogram\}}}$$

$$\Rightarrow \frac{2.9}{25839.574} = 1.12 \times 10^{-04} = .000112$$

Concentration of cypermethrin in the sample = Response factor x sample peak response in µg/ml or mg/l { calculated from chromatogram}

Formula 2 (Wang, 2005)

$$\text{Response Factor of cypermethrin Standard} = \frac{\text{Standard peak response (from chromatogram)}}{\text{Standard concentration}}$$

$$\Rightarrow \frac{25839.574}{2.9} = 8910.197$$

$$\text{Concentration of the pesticide in the sample In mg/ml} = \frac{\text{SamplePeakResponse (from chromatogram)}}{\text{Standard response factor}}$$

Chlorpyrifos

Formula 1

$$\text{Response Factor of Chlorpyrifos Standard} = \frac{\text{Concentration of Standard}}{\text{Response or Peak area of Standard \{Calculated from chromatogram\}}}$$

$$\Rightarrow \frac{8.3}{468236.625} = 1.77 \times 10^{-05}$$

Concentration of chlorpyrifos in the sample = Response factor of standard x sample peak response in µg/ml or mg/l { calculated from chromatogram}

Formula 2

Response Factor of chlorpyrifos Standard= $\frac{\text{Standard peak response(from chromatogram)}}{\text{Standard concentration}}$

$$\Rightarrow \frac{468236.625}{8.3} = 56414.05$$

Concentration of the pesticide in the sample=$\frac{\text{Sample Peak Response(from chromatogram)}}{\text{Standard response factor}}$ In mg/ml

Profenofos

Formula 1

Response Factor of Profenofos Standard = $\frac{\text{Concentration of Standard}}{\text{Response or Peak area of Standard {Calculated from chromatogram}}}$

$$\Rightarrow \frac{8.3}{1238613} = 6.7 \times 10^{-07}$$

Concentration of profenofos in the sample = Response factor of standard x sample peak response in µg/ml or mg/l { calculated from chromatogram}

Formula 2

Response Factor of profenofos Standard = $\frac{\text{Standard peak response (from chromatogram)}}{\text{Standard concentration}}$

$$\Rightarrow \frac{12386139.0}{8.3} = 1492305.904$$

Concentration of the pesticide in the sample=$\frac{\text{Sample Peak Response(from chromatogram)}}{\text{Standard response factor}}$ In mg/ml

Table 4.2.3.1 The m/z and emerging time of pesticides under study

Pesticides	m/z	Chemicalformula & Mol Wt	Pesticides metabolites	Retention time	Reference
Cypermethrin (RS)-cyano(3-phenoxyphenyl)methyl (1RS)- *cis-trans*-3-(2,2-dichloroethenyl)-2,2 dimethyl-s s cyclopropane carboxylate (Pyrethroid compound)	109, 163,165, 209	$C_{22}H_{19}Cl_2NO_3$, MW= 416.32	4-hydroxy 3-phenoxybenzoic acid, PBA, alpha cyano-3-phenoxybenzyl alcohol, 3-phenoxybenzyl alcohol, 3 phenoxy methyl benaldehyde, DCVA.	26.36 min	Bolygo (1990)
chloropyriphos (3,5,6 trichloro 2 pyridinol phophorothioate. An organophosph-ate compound)	97, 258, 125	$C_9H_{11}Cl_3NO_3PS$ MW 350.59	3,5,6 trichloro-2pyridinol, 3,5,6 trichloro- 2pyridyl phosphate, des-mono-ethyl-chloropyriphos, des-ethyl-chloropyriphos.	18.48 min	Bolygo (1990), Elzerman and Paddock (2000)
Profenofos(o-(4-bromo-2-chlorophenol)-o-ethyl-s-n-propyl phosphorothiote-An Organophosp-hate compound)	63, 142, 144, 206, 208, 210	$C_{11}H_{15}BrClO_3PS$ MW= 373.6	o-(4-bromo-2-chlorophenol)-o-ethyl phosphorothiote, o-(4-bromo-2-chlorophenol)-o-ethyl phosphate,4-bromo-2-chlorophenol,4-bromo-2-chlorophenol glucoronide, 4-bromo-2-chlorophenol sulphate.	20.53 min	Whitney (2007) Sugitate and Kana (2008)
Imidacloprid 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine. (Neonicotinoid compound)	256,209,175	$C_9H_{10}ClN_5O_2$ MW 255.6	monohydroxy imidacloprid, imidacloprid guanidine, imidacloprid urea, imidacloprid nitrosamine, imidacloprid olefin, 6 chloronicotinic acid, 6 chloronicotinic acid aldehyde, 6 hydroxy nicotinic acid aldehydes, 6 hydroxy nicotinic acid.	24.42 min	Renata et al. (2008) Lau et al.(2008)

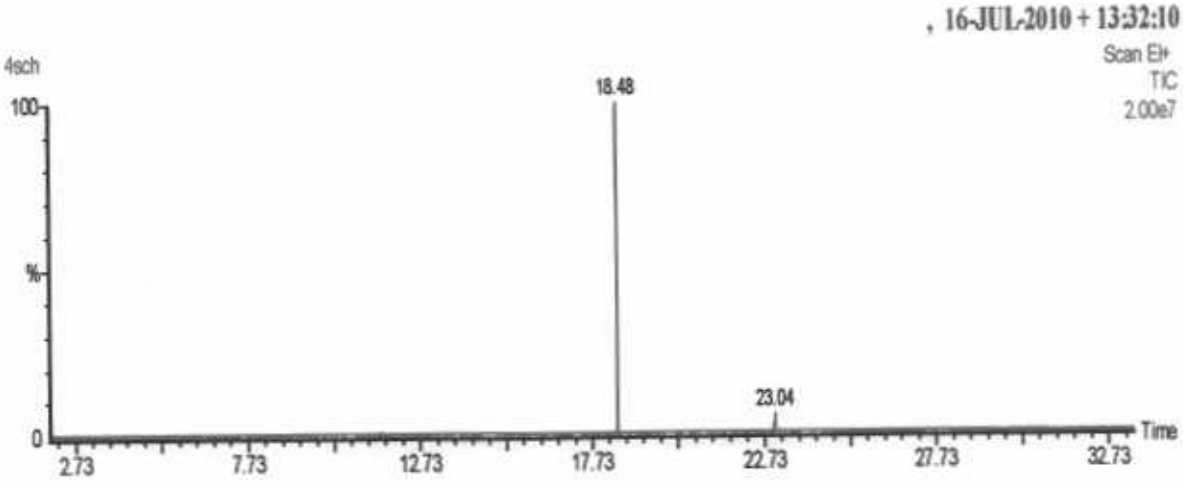
4.2.4. Mean pesticide concentration of whole cow milk

4.2.4.a. Total ion Chromatogram (TIC) of standards of pesticides

Figures 4.2.4.1 to 4.2.4.4 show the chromatograms of analytical standards of chloropyriphos, cypermethrin, profenophos and imidacloprid. The peaks of the standards were identified from m/z ratio of the pesticides as appeared on GC-MS chromatogram. However, the confirmation of the same compound was carried out from NSIT GC-MS library search of same molecular weight, chemical formula and composition.

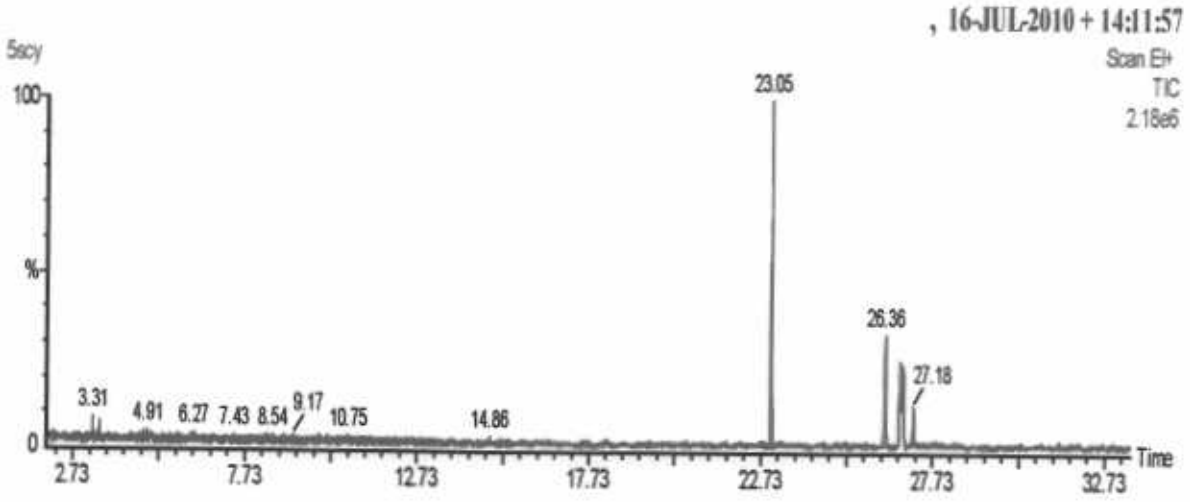
Chlorpyriphos (3,5,6 trichloro 2 pyridinyl phophorothioate) belongs to organophosphate group of insecticides. It's chemical formula is $C_9H_{11}Cl_3NO_3PS$ and molecular weight is - 350.59 g. M/Z's of chloropyriphos, its precursors & metabolites are 97, 125, 197, 258, 286, 302, 314 and that of quantitation ion is 314 whereas the retention time of chlorpyriphos on GCMS chromatogram is 18.48 min. It's main metabolites are 3,5,6 trichloro- 2pyridinol, 3,5,6 trichloro- 2pyridyl phosphate, des-mono-ethyl-chloropyriphos, des-ethyl-chloropyriphos.

Figure 4.2.4.1. Chromatogram showing retention time of Chloropyriphos standard
Retention time, 18.47, 18.48 min for m/z 97
m/z of chloropyriphos & metabolites, 97, 125, 258



Cypermethrin, (RS)-cyano (3 phenoxyphenyl) methyl(1RS)- *cis-trans*-3-(2,2-dichloroethenyl)-2,2 dimethyl-s s cyclopropane carboxylate, belongs to pyrethroid group. It's molecular weight is 416.3 g and chemical formula is $C_{22}H_{19}Cl_2NO_3$ g. The m/z's of cypermethrin, it's precursors and metabolites are 109,125,163 whereas the retention time on GCMS chromatogram is 26.36 min. It's main metabolites are 4-hydroxy 3-phenoxybenzoic acid, PBA, alpha cyano-3-phenoxybenzyl alcohol, 3-phenoxybenzyl alcohol, 3 phenoxy methyl benaldehyde, DCVA.

Figure 4.2.4.2 Chromatogram showing retention time of Cypermethrin standard
Retention time 26.34, 26.36, 26.81, 26.86, 27.16, 27.21, 27.22, 27.24, min
m/z of cypermethrin & metabolites- 163, 165, 181, 209

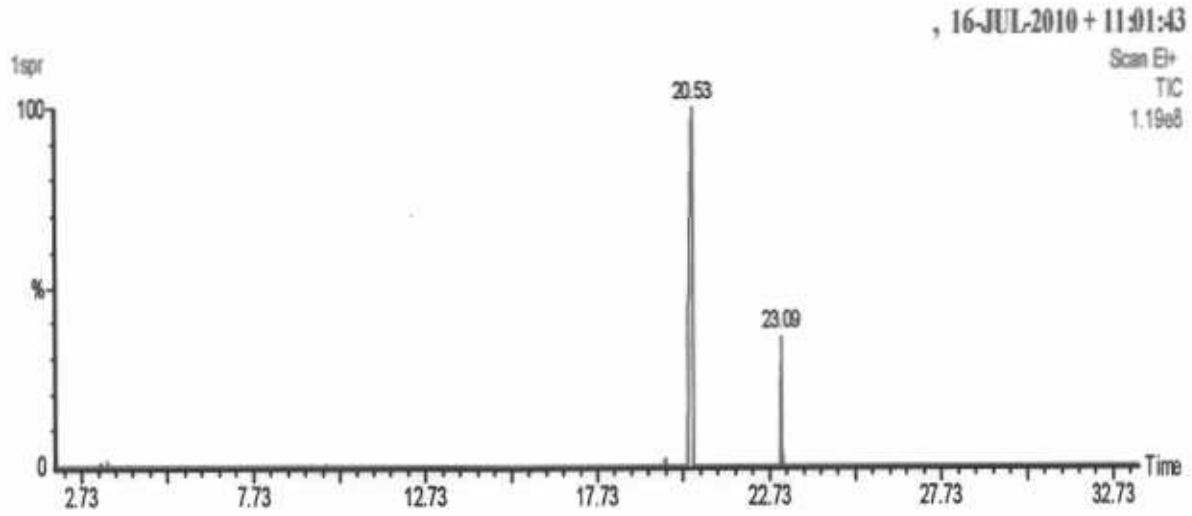


Profenophos (o-(4-bromo-2-chlorophenol)-o-ethyl-s-n-propyl phosphorothiote) belongs to organophosphate group with chemical formula of $C_{11}H_{15}BrClO_3PS$ and molecular weight of 373.63g. The m/z's of profenophos, its precursors and metabolites are 208,267,279 and 286 and that of quatitation ion is 337 whereas the retention time on GCMS chromatogram is 20.53 min. The main metabolites are o-(4-bromo-2-chlorophenol)-o-ethyl phosphorothiote, o-(4-bromo-2-chlorophenol)-o-ethyl phosphate, 4-bromo-2-chlorophenol, 4-bromo-2-chlorophenol glucoronide, 4-bromo-2-chlorophenol sulphate.

Figure 4.2.4.3. Chromatogram showing retention time of Profenophos standard

Retention time. 20.44, 20.52, 20.53, min

M/Z of profenophos & metabolites. 63, 142, 144, 206, 208, 210



Imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine is a neonicotinoid with chemical formula of $C_9H_{10}ClN_5O_2$ and molecular weight of 255.66 g. The m/z's of imidachloprid, its precursors and metabolites are 256/258,278/280,290,305/308,287/296 and the retention time on GCMS chromatogram is 24.42 min. It's main metabolites are monohydroxy imidacloprid, imidacloprid guanidine, imidacloprid urea, imidacloprid nitrosamine, imidacloprid olefin, 6 chloronicotinic acid, 6 chloronicotinic acid aldehyde, 6 hydroxy nicotinic acid aldehydes, 6 hydroxy nicotinic acid. However imidachloprid was not detected in any sample because it cannot be analyzed by GC-MS..

Figure 4.2.4.b.1: Chromatogram TIC of raw milk showing retention time of chlorpyrifos

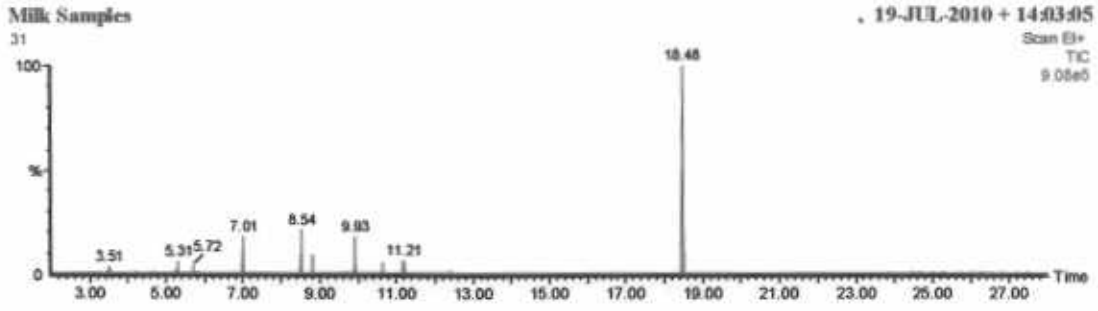
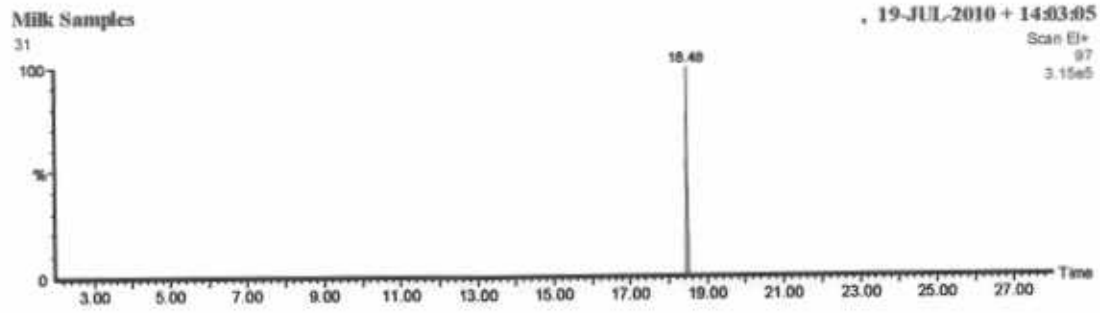
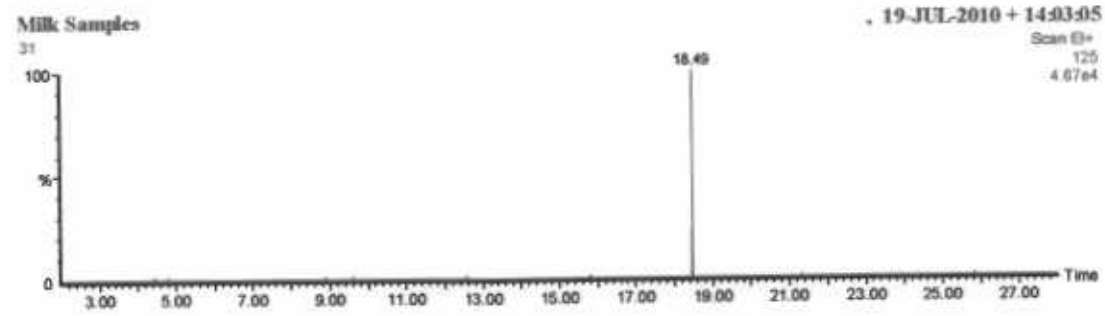


Figure 4.2.4.b.2 a -c: Chromatogram TIC of chloropyriphos metabolites m/z 97, 125, 258 with retention time 18.48, 18.49 mins.

a.



b.



c.

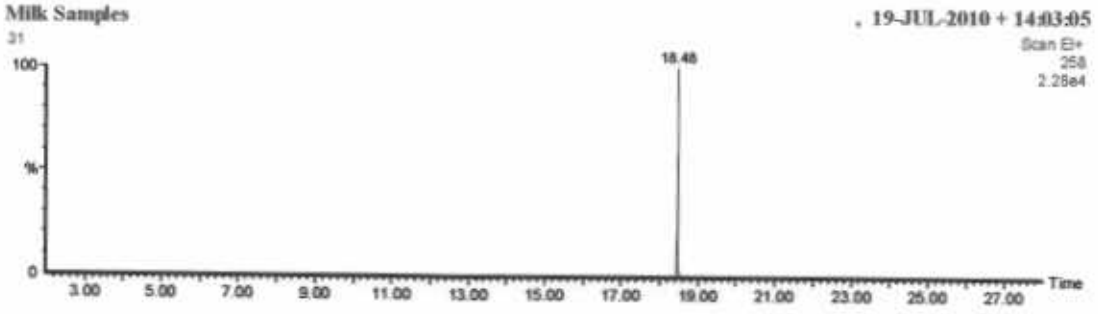
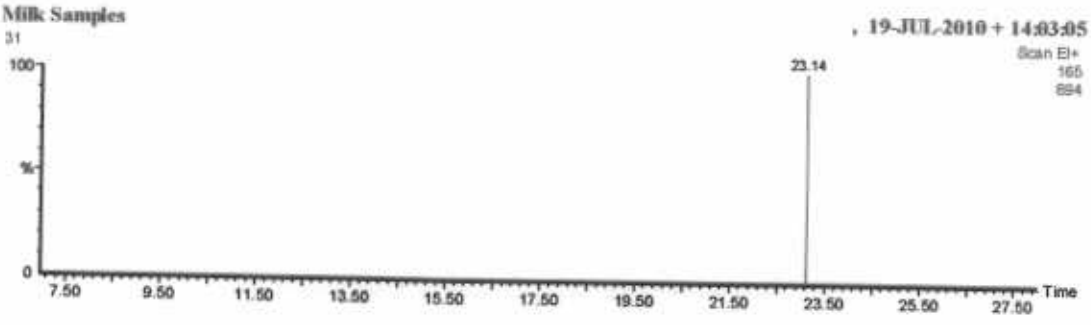


Figure. 4.2.4.b.3. a-b. Chromatogram TIC showing retention time of cypermethrin, it'sprecursor's & metabolite m/z 165, 109 is 23.14 , 26.88 min

a.



b.

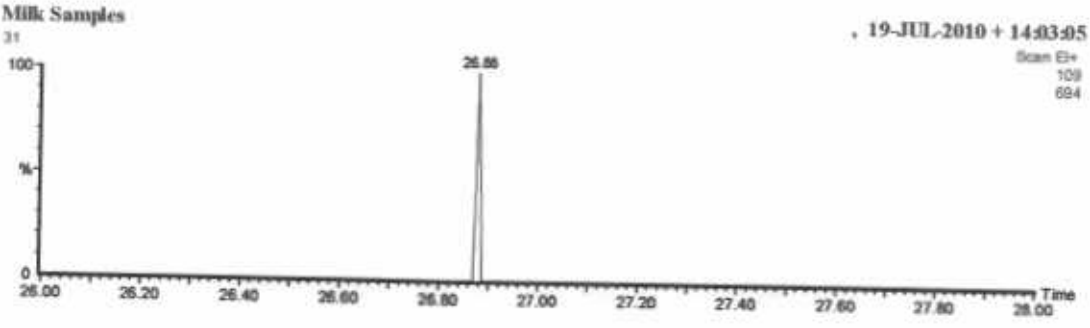


Figure 4.2.4.b.4: Chromatogram TIC of raw milk showing retention time of profenofos

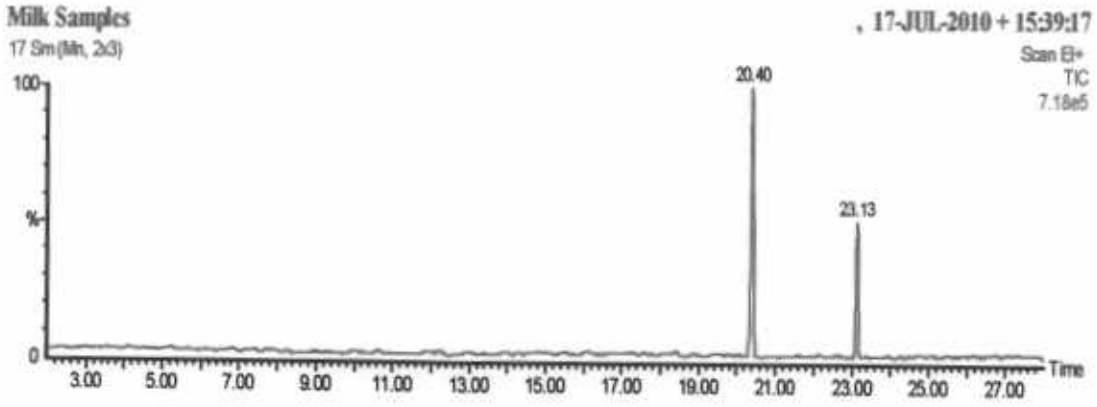
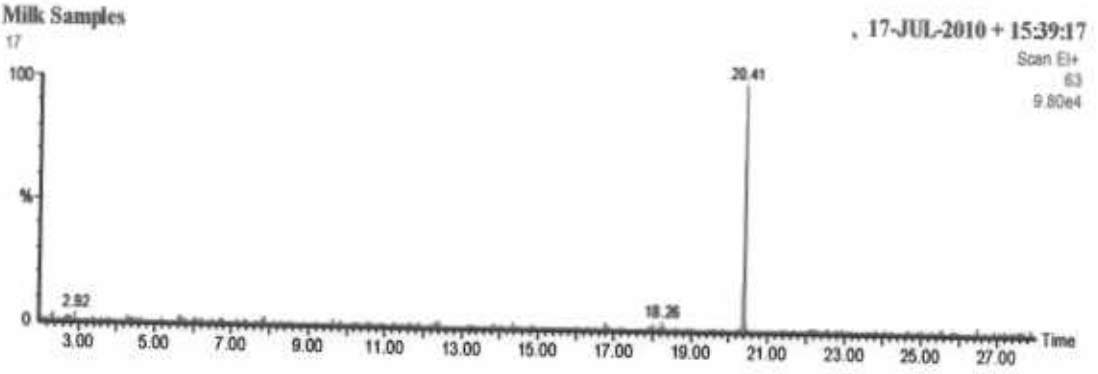
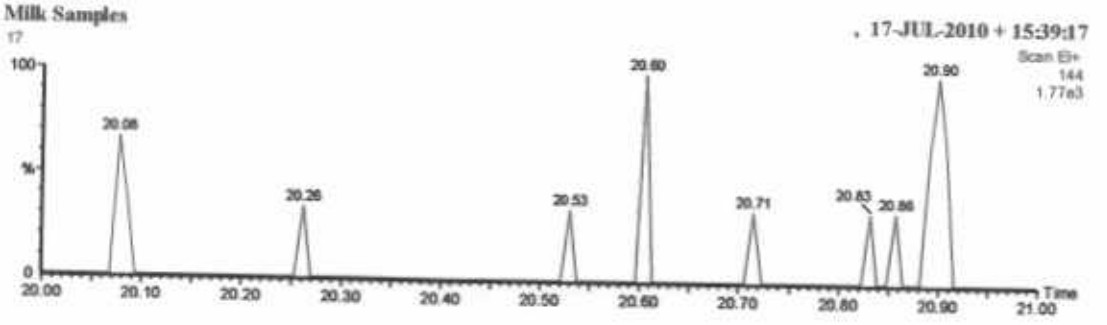


Figure 4.2.4.b.4 a-e : Chromatogram TIC of raw milk showing retention time of profenophos metabolites m/z 63, 144, 206, 208, 210.

a.



b.



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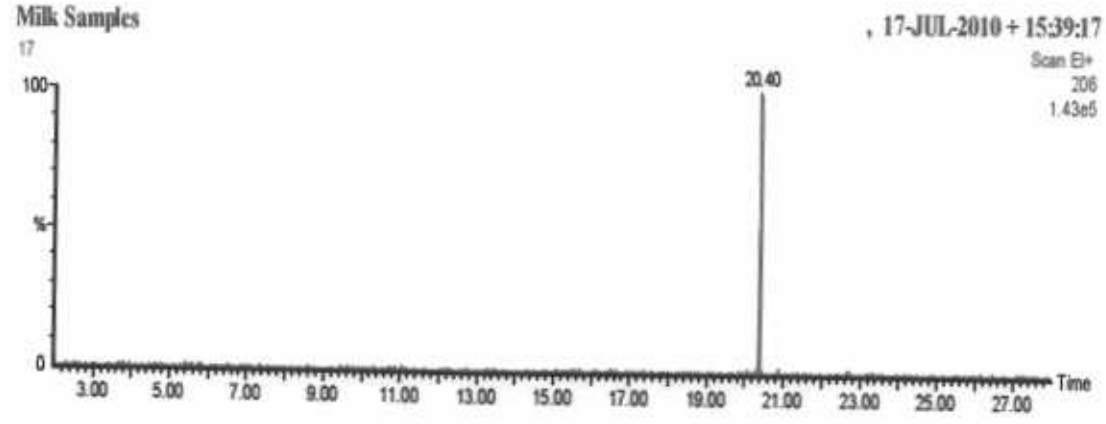
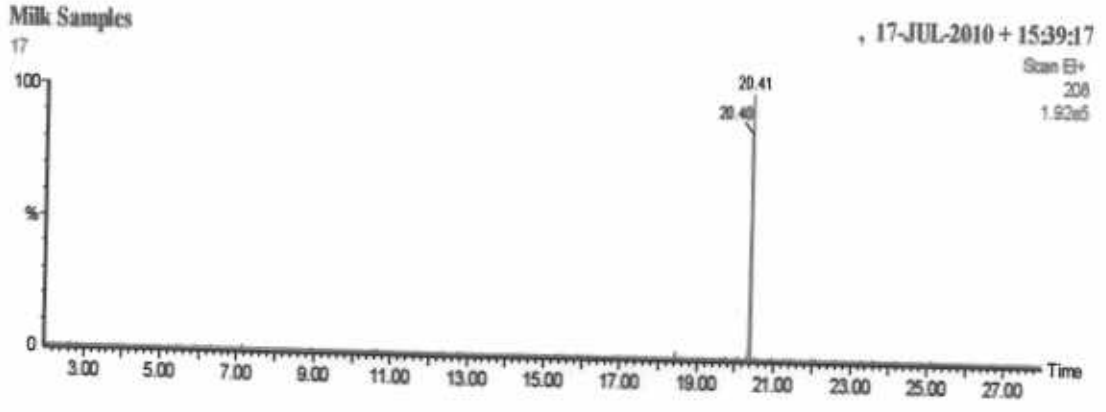

Milk Samples
17
, 17-JUL-2010 + 15:39:17
Scan EI+
206
1.43e5
20.40
100
%
0
3.00
5.00
7.00
9.00
11.00
13.00
15.00
17.00
19.00
21.00
23.00
25.00
27.00
Time

d.


Milk Samples
17
, 17-JUL-2010 + 15:39:17
Scan EI+
206
1.92e5
20.41
20.40
100
%
0
3.00
5.00
7.00
9.00
11.00
13.00
15.00
17.00
19.00
21.00
23.00
25.00
27.00
Time

e.

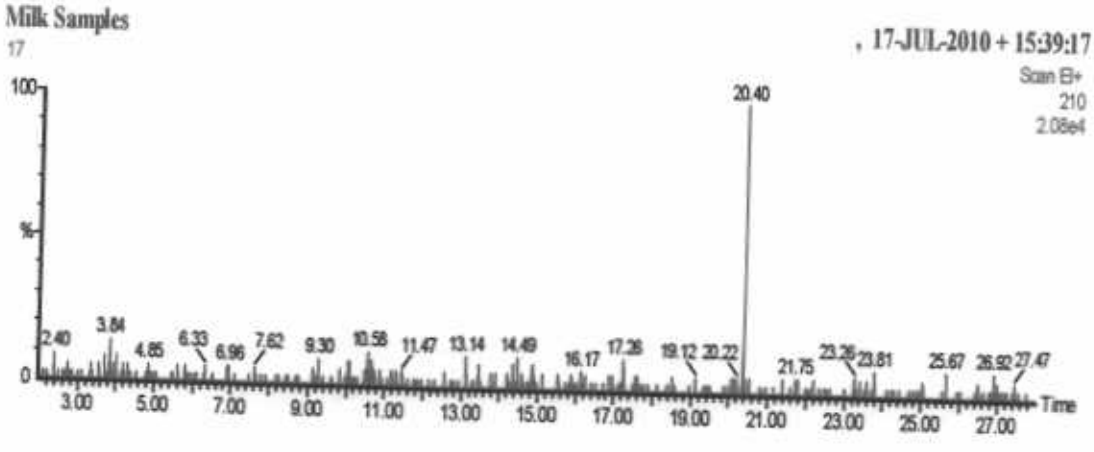
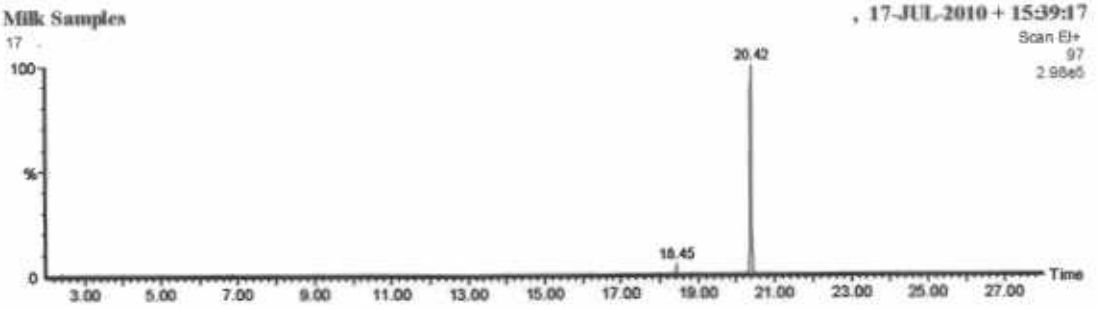

Milk Samples
17
, 17-JUL-2010 + 15:39:17
Scan EI+
210
2.08e4
20.40
100
%
0
2.40
3.84
4.85
6.33
6.96
7.62
9.30
10.58
11.47
13.14
14.49
16.17
17.26
19.12
20.22
21.75
23.26
23.81
25.67
26.92
27.47
3.00
5.00
7.00
9.00
11.00
13.00
15.00
17.00
19.00
21.00
23.00
25.00
27.00
Time

Figure 4.2.4.b.5 a-b: Chromatogram TIC of raw milk showing retention time of profenofos and it's metabolites m/z 97, 258

a.



b.

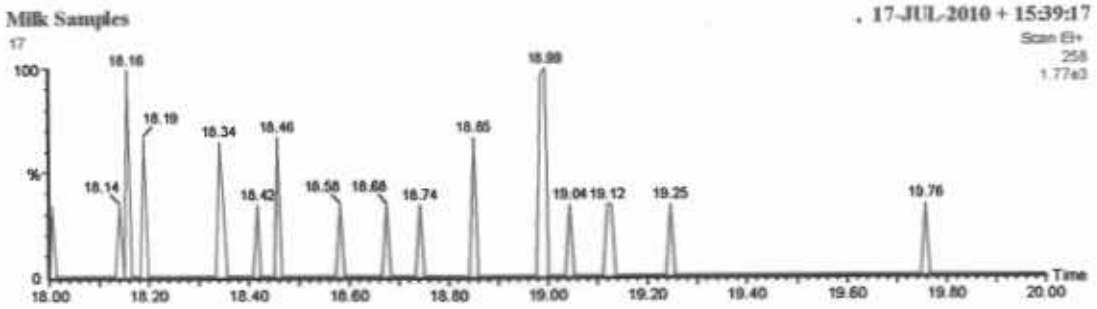
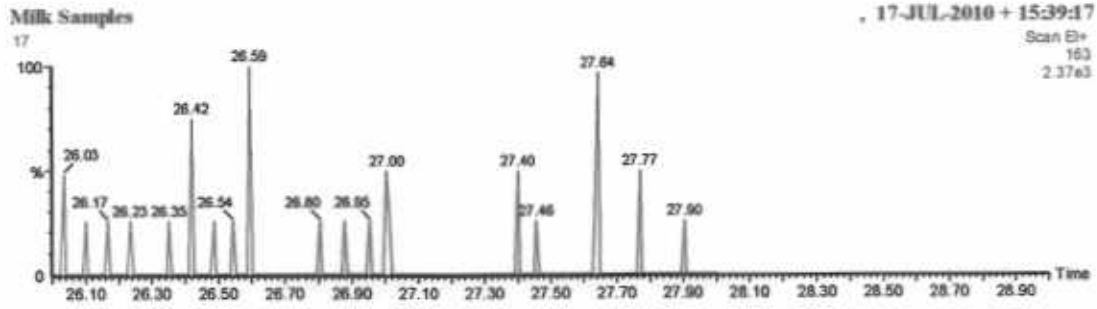
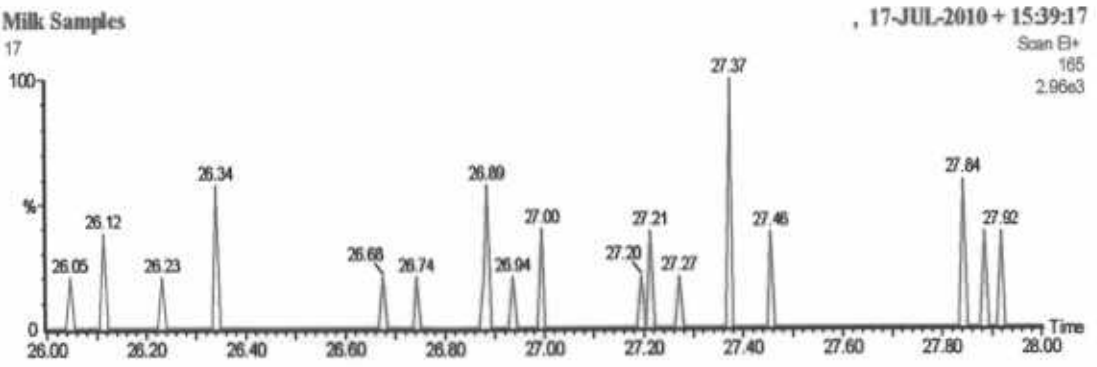


Figure 4.2.4.b.6a-c : Chromatogram TIC of raw milk showing retention time of cypermethrin and it's metabolites m/z 163, 165, 209

a.



b.



c.

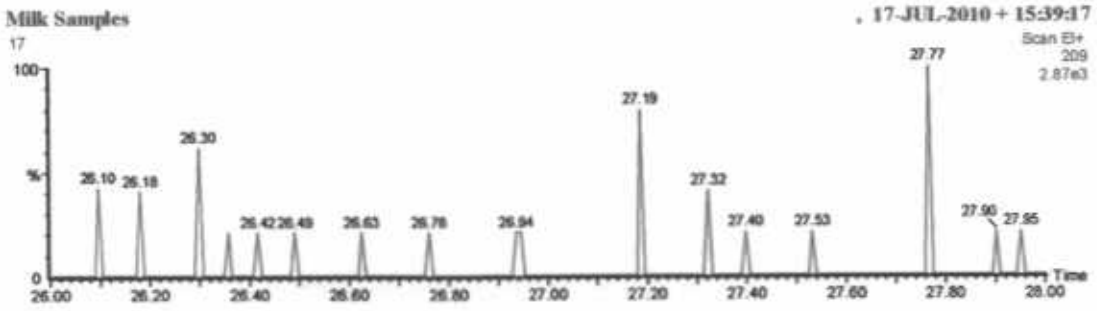


Figure 4.2.4.b.7 : Chromatogram TIC of raw milk showing retention time of organo phosphorus and it's metabolites.

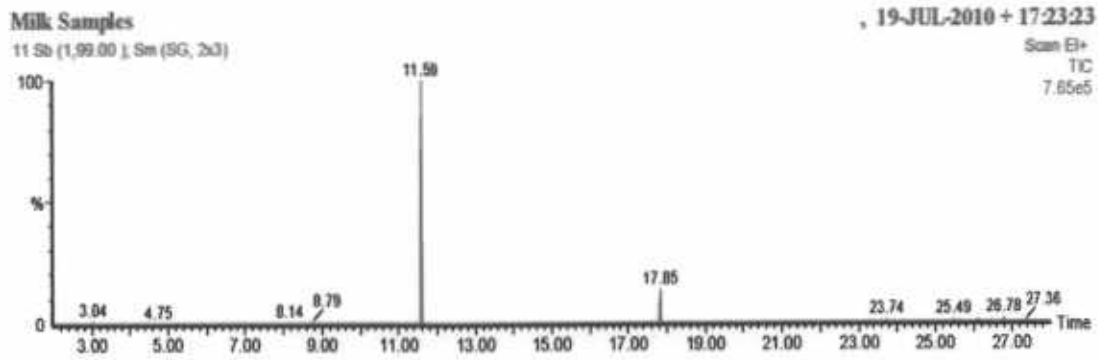


Figure 4.2.4.b.7 a : Mass scan of raw milk showing fragmentation pattern of chlorpyrofos

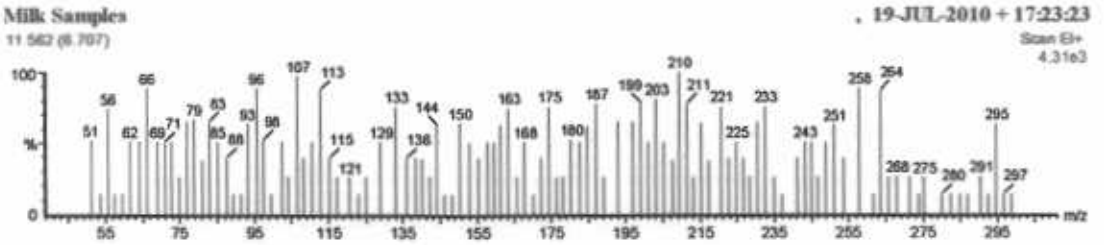


Figure 4.2.4.b.8 : Chromatogram TIC of raw milk showing retention time of cypermethrin

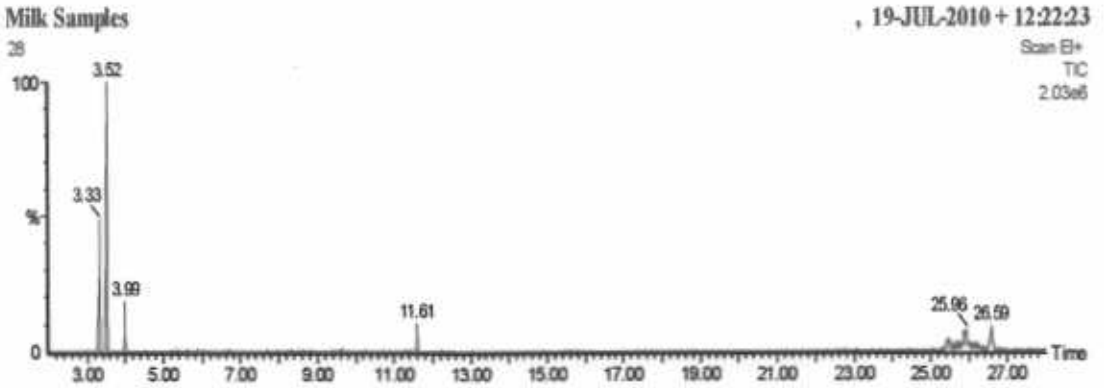


Figure 4.2.4..b.9. Total ion chromatogram (TIC) of milk sample showing cypermethrin

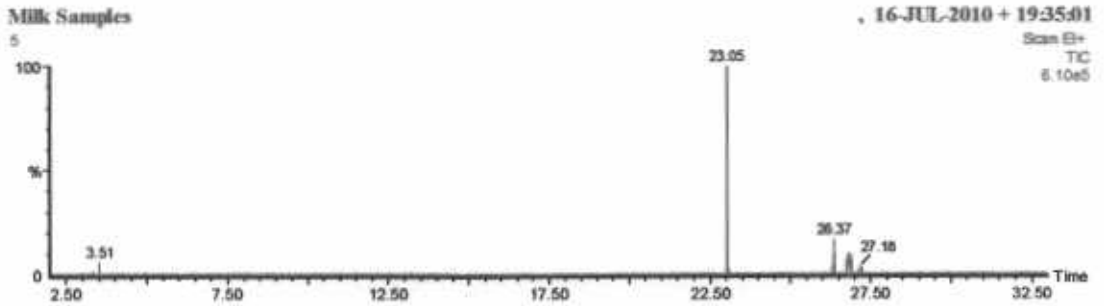
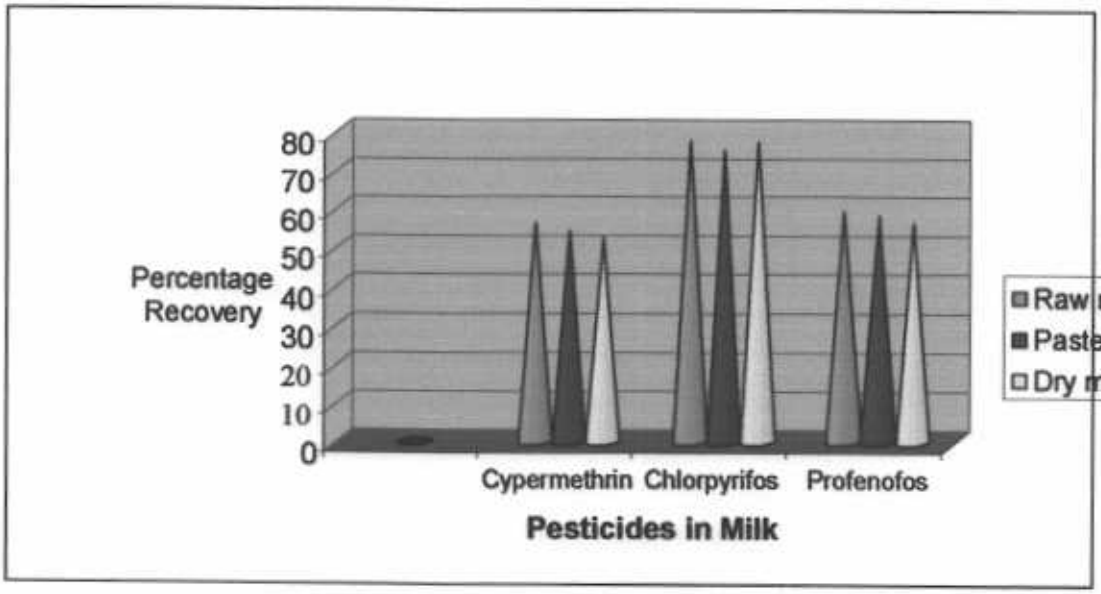


Figure 4.2.4.10. shows the recovery rate of various pesticides in milk. It shows that the recovery rate of Cypermethrin, Chlorpyrifos and Profenofos in raw milk is 56.25%, 78.12% and 59.4%, in pasteurized milk is 54 %, 75.35 % & 58.65 % while in dry milk is 52.67 %, 77.58 % and 56.33 % respectively whereas in diet is 100%.

Figure 4.2.4.10. Recovery Rate of pesticides in Different Types of Milk



The average content of cypermethrin, chlorophyriphos, profenophos, imidacloprid and emamectin in the dairy milk of dairy cattle farmed in the urban and rural sites within Peshawar district is presented in Table 4.2.4.1. Analysis of GCMS chromatograms of dairy cattle milk samples from urban and rural farms showed that approximately similar amounts of cypermethrin and chloropyriphos appeared on the chromatograms of urban farm 1. The main differences between urban and rural farms in the pattern of appearance of pesticides were that low amounts of all of them were found in rural farm 5 than other rural and urban farms. Greater concentrations of cypermethrin and profenophos were noted in rural farm 4 whereas chloropyrifos appeared to be high in urban farm 1 followed by other farms. When the concentrations of cypermethrin, chloropyrifos and profenofos

in the milk samples of any of the farm was compared it was noted that more or less same amounts of cypermethrine and chloropyrifos were obtained from all farms whereas the concentrations of profenofos was low in all farms.

Table 4.2.4.1: Average content (mg L^{-1}) of pyrethroid, and organo-phosphate groups of pesticides in the dairy milk of rural and urban farms

Farms No	Sites	Groups		
		Pyrethroid	Organo phosphate	
		Pesticides		
		Cypermethrin	Chlorpyriphos	Profenophos
1	Urban	0.221c	0.228c	0.00152b
2	Urban	0.208c	0.029a	0.0006a
3	Urban	0.112b	0.018a	0.00144b
4	Rural	0.321d	0.081b	0.00212c
5	Rural	0.02a	0.107b	0.00156b

*means (n=3) showed by different letters within columns are significantly different at the 5% level of probability

Figure 4.2.4.11. Comparison between pesticide level of different farms

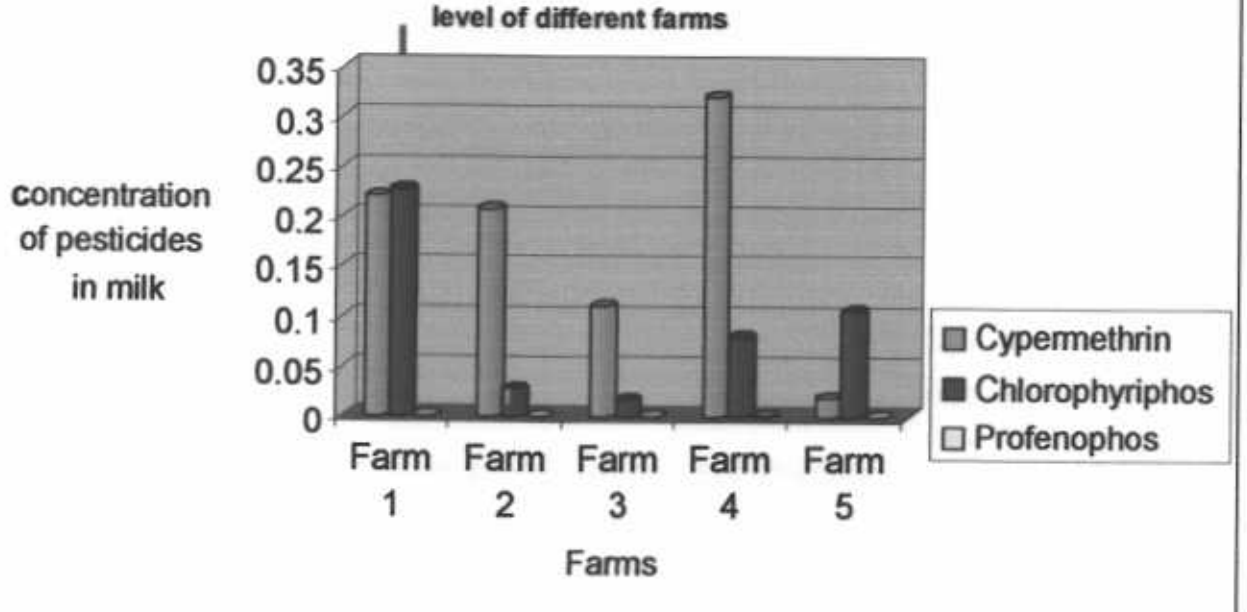


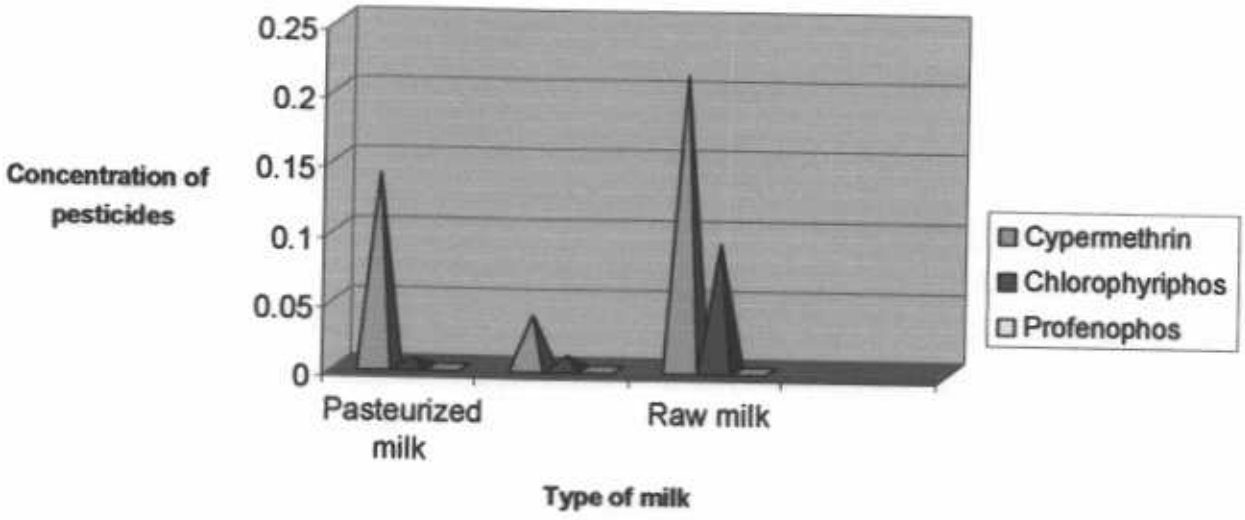
Table 4.2.4.2. shows the difference in the average content of cypermethrin, chlorophyriphos and profenophos in the 3 types of milk. There was significant difference in the content of cypermethrin in all three types of milk whereas there was a trend that significantly greater ($p<0.05$) content of cypermethrin was noted in the raw milk than in pasteurized and dry milk. Nevertheless there was no significant difference in the content of chlorophyrifos and profenophos between pasteurized and dry milk. Nonetheless, significantly greater ($p<0.05$) content of chlorophyrifos and profenophos was noted in the raw milk than pasteurized and dry milk. Imidacloprid and emamectin was not undetectable from GCMS of any of the milk.

Table 4.2.4.2. The average content (mgL^{-1}) of pyrethroid and organo-phosphate groups of pesticides in the pasteurized, dry and raw milk.

	Pyrethroid	Organo phosphate	
	Pesticides		
	Cypermethrin	Chlorophyriphos	Profenophos
Pasteurized milk	0.176b	0.0926	9.8×10^{-4a}
Dry milk	0.039a	0.0099a	5.7×10^{-4a}
Raw milk	0.212c	0.092b	6.8×10^{-3b}

*means (n=3) showed by different letters within columns are significantly different at the 5% level of probability

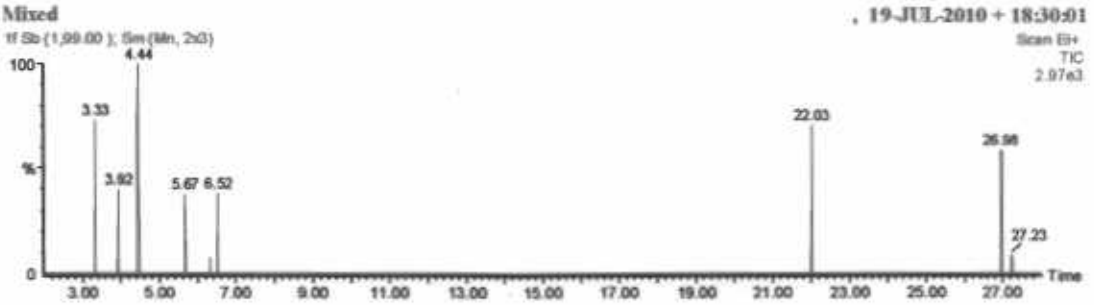
Figure 4.2.4.11.: Comparison between pesticide level in raw, pasteurized and dry milk



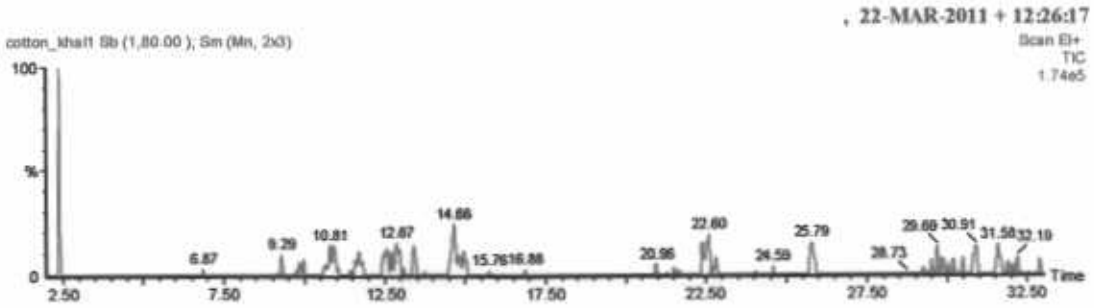
4.2.4.b. Total ion Chromatogram (TIC) of pesticides in animal fodder

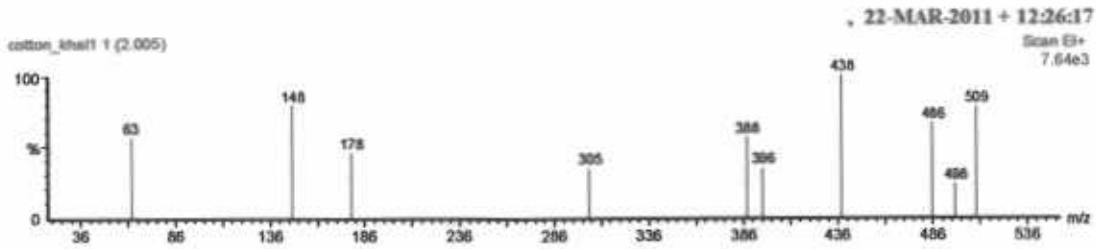
Figure 4.2.4.b.a - c. Chromatogram TIC and mass scan of animal feed/fodder showing retention time of pesticides and their metabolites.

a.



b.





c.

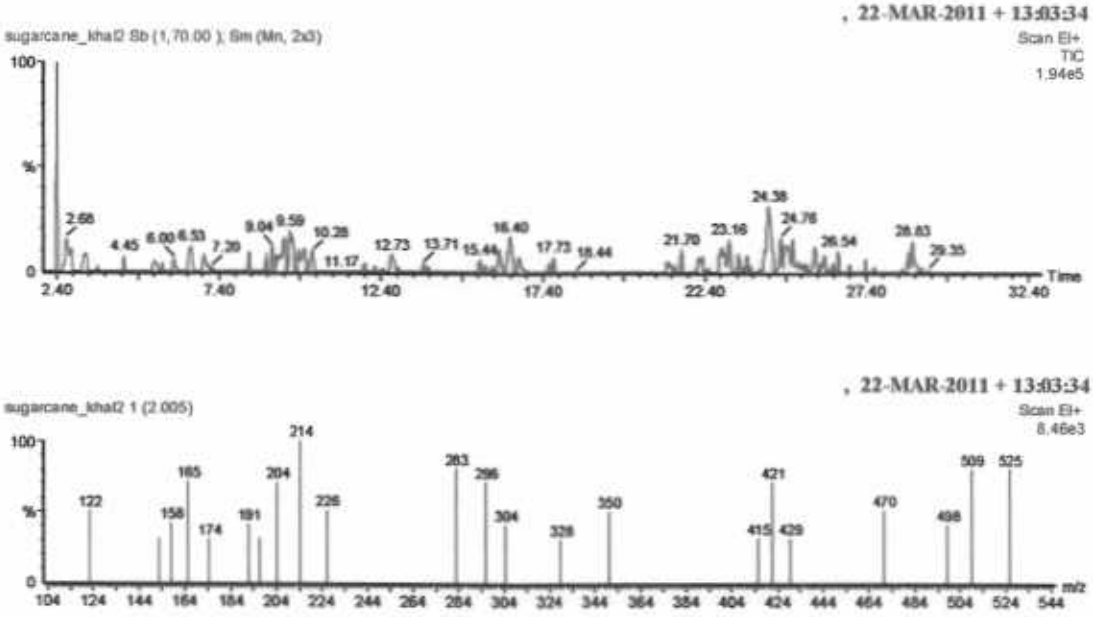


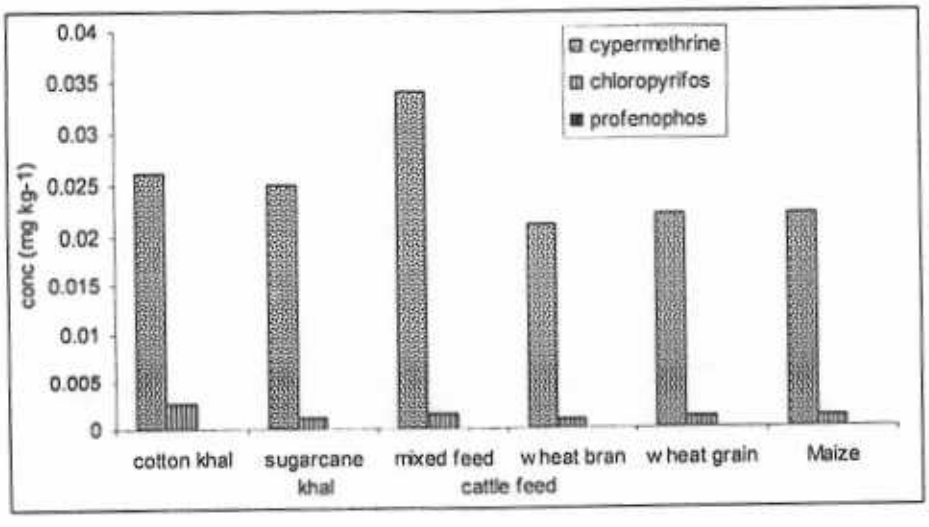
Table 4.2.4.b.1. shows the average content of cypermethrin, chlorpyriphos and profenophos in the diet of dairy cattle farmed in urban and rural areas. The result shows that the main difference in the amounts of cypermethrin, chlorpyrifos and profenophos was that the low content of profenophos was extracted from all feeds followed by chlorpyriphos and cypermethrin. It was noted that approximately same amounts of cypermethrin was found in all types of dairy cattle feed whereas significantly greater ($P<0.05$) content of chlorophyrifos was noted in cotton khal and low content was extracted from wheat bran and intermediate amount from all other feeds. Profenophos was low in wheat bran and wheat grain than other four feeds. Imidacloprid was extracted from cotton khal than was not detected in any of the other dairy csattle feeds. Emamectin was not found in any of the diet.

Table 4.2.4.1. Comparison between average pesticides level in different types of diets

	Pyrethroid (mg/L)	Organo phosphate (mg/L)	
	Pesticides		
Type of feed	Cypermethrin	Chloropyriphos	Profenophos
Cotton Khal	0.026	0.00269c	7.6×10^{-4}
Sugar Cane Khal	0.025	0.0012b	7.9×10^{-4}
Mixed feed	0.034	0.0016b	9.8×10^{-4}
Wheat bran	0.021	0.0009a	6.6×10^{-4}
Wheat grain	0.022	0.0011b	6.2×10^{-5}
Maize	0.022	0.0013b	5.9×10^{-5}

*means (n=3) showed by different letters within columns are significantly different at the 5% level of probability

Figure 4.2.4.b.d. Comparison between average pesticides level in different types of diets



4.2.5. Comparison between the mean content of pesticides of present study with the world reported data.

Pesticide	Present study	Pagliuca (2006) mg kg^{-1}	Salas et al (2003) mg kg^{-1}	Bordet (2002) mg kg^{-1}	Roothwell et al (2001) mg kg^{-1}	Chen et al (1997) mg kg^{-1}
Cypermethrin	0.21	-	-	-	0.015	<0.0 19
Chlorpyrifos	0.092	-	-	0.026-0.045	-	-
Profenofos	$6.8x10^{-3}$	0.005-0.018	0.005-0.020	-	-	-
Imidacloprid	NA	-	-	-	-	-

4.2.6. Comparison between the mean content of pesticides of present study with the maximum residue limits.

	Present study			MRL	Present study	MRL
Pesticide	Raw milk mg L^{-1}	Pasteurized milk mg L^{-1}	Dry milk mg L^{-1}	MRLs mg L^{-1} (Pesti.Residues MRL ,2008 ; Sassine 2003)	Fodder mg kg-1	MRLs mg kg^{-1} (MRL standard Aust. Govt, 2012)
chloropyriphos	0.092	0.092	0.009	0.01 -0.02	0.0009-0.002	30 (cotton fodder)
Cypermethrin	0.21	0.17	0.04	0.05 / 0.01	0.021-0.034	10 (cotton fodder)
Profenophos	6.8×10^{-3}	9.8×10^{-4}	$5.7x 10^{-4}$	0.01	9.8×10^{-4} - 5.9×10^{-5}	1(cotton fodder)
Imidacloprid	ND	ND	ND		ND	2 (sugarcane fodder)

Chapter 5
DISCUSSION

5.0. DISCUSSION

5.1. Heavy Metals

The objective of this study was to evaluate the impact of environmental pollution associated with the increase in urbanization and industrialization on dairy milk quality in terms of it's toxic metal and pesticide content and also assess forage induce toxicity of these in milk. Increase in industrialization and urbanization poses severe impact on human health. As a consequence of urbanization several tons of domestic, municipal and hazardous waste is dumped into rivers, canals and irrigation channels. These wastes contain elevated levels of organic and inorganic compounds. Wastewater consumed by cattle usually contains threshold levels of some of the environmentally toxic elements such as Cd and Pb and other elements like Cu, Ni, Co and Cr. Dairy cattle farmed in urban areas are also under constant threat because of exhausts from heavy traffic, air pollution and hazardous waste, than those farmed in rural areas. The toxic substances from these exhausts and air settle on the grazing grounds which also receive toxic metals and pesticides due to irrigation from waste water.

In this study Cadmium (Cd) and Copper (Cu) were found in 100% of the samples in moderate concentrations. Chromium (Cr), Cobalt (Co), Nickel (Ni) and Lead (Pb) were found in 50-60% of the samples. Cr was found in low concentration in dairy milk samples of all the farms but comparatively higher levels were noted in the dairy milk of urban farm 1 than any of the rural or urban farms. Cd, Cr, Co, Cu and Ni levels in this study were significantly greater ($p<0.05$) in urban farms than the rural farms (Table 4.1.4.1.). There was no significant difference in the amount of Pb in any of the urban or rural farms but comparatively higher levels were found in the dairy milk of urban farm 1

compared to other urban and rural farms. All the elements were higher in urban farm I as compared to all the other farms. The difference in the content of heavy metals in dairy cattle milk between urban and rural farms is most likely because urban farms are located in the centre of densely populated towns and are more exposed to air, water and soil contamination from disposal of untreated industrial and hazardous waste than rural farms. The result of this study is in agreement with the findings of Simsek et al. (2000). They reported that Cu content in the dairy cattle milk farmed in urban area near the pharmaceutical industrial unit was 0.96 mg L^{-1} and was 0.58 mg L^{-1} in the dairy cattle milk farmed near the road than rural areas. They concluded that greater content of Cu in the dairy cattle milk of urban areas was more likely because of dumping of untreated industrial effluents to the soil and water. Cattle are more vulnerable to Cu contamination because of drinking and ingesting Cu contaminated water and soil. In another study Debashis (2009) reported that Pb content in milk samples collected from urban farms near industrial processing units was greater than that of milk samples of rural farms.

In this study there was no difference in the Pb content between urban and rural farms and although Pb was not found in all the farms but it was in very high concentration in some of the farms. This revealed the contention that Pb contamination in milk is the most common problem in both farms. This suggests ingesting Pb-contaminated soils by dairy cattle in both farms. Pb contamination in milk is widely reported in the previous literature. In another study Bahatia and Choudhry (1996) reported that Pb content was 7.20 mg L^{-1} in the dairy cattle milk farmed in urban area located near the road. Swarup et al. (2005) reported that Pb content was significantly greater ($p<0.05$) in urban milk samples than rural milk samples. They concluded that the difference in the Pb content

between urban and rural farms is most probably because of urbanization and exhausts from traffic. Dwivedi et al. (2001) reported that Pb and Cd content ranged from 0.75 and 0.05 mg L^{-1} in the urban milk sample. They concluded that urban farm was located near Pb-Zn smelter and because of ingesting Pb contaminated soil, Pb and Cd contamination was noted in cattle milk.

The result of this study resemble the findings of Jan et al. (2011). They reported that greater content of Cu, Cr, Ni and Pb in the dairy milk farmed in urban areas near the road and industrial areas was greater than that of rural areas, most probably because of drinking wastewater and ingesting Pb-contaminated soil. They studied the effect of industrial wastewater on the quality of dairy milk and dairy milk products within Peshawar and noted that the mean content of Cr, Cu, Ni and Pb was two folds greater in the milk of dairy cattle drinking wastewater than drinking clean water. They concluded that the greater content of Cr and Ni is because of discharge of industrial wastewater into rivers and irrigation channels whereas elevated levels of Cu and Pb in dairy cattle milk is most probably because of corrosion of water carrying pipelines and exhausts from heavy traffics. They found out that the concentration of Cu, Cr, Ni and Pb in dairy milk were 14.88, 8.44, 0.30 and 13.23 ug L^{-1} respectively.

The effect of toxic metal content in dairy cattle diet on milk quality is estimated through comparison between metal content in the dairy milk of this study with maximum residue limits of Cd, Co, Cr, Cu, Ni and Pb in dairy cattle milk as reported by Muzzarelli et al. (1988), AOAC (1989) and Lewinsky (2007). The results of this study show that the mean content of Cd, Cr, Cu, Co, Ni and Pb were 86, 24, 12, 14, 63 and 89 times greater respectively in raw dairy milk than maximum residue limits (JECFA 1989) for the same

elements in normal milk. Pennington et al. (1987), Varo et al. (1980), Fox et al. (1997), Solis et al. (2009) reported that trace elements content was greater in cow's milk samples than maximum allowable limits for cow's milk. They concluded that trace elements contamination in milk was most probably because of increase in urbanization, air borne pollution and industrialization. The average chromium content in commercial dairy milk was reported by a Chinese study as 0.17 ± 0.05 mg kg^{-1} and by a Japanese study as 0.09 ± 0.03 mg kg^{-1} (Qin et.al. 2009).

The level of essential nutrients and toxic metals in milk primarily depends on their content in the diet. Cattle diet with elevated levels of these elements causes an increase in their level in milk also. Considering the mean concentrations of metals in different types of animal feed in our study, it appears that the concentration of chromium is significantly high in all types of animal diets except wheat bran. Copper is very high in cotton khal (83.3 mg kg^{-1}) and comparatively high in sugarcane khal (5.4 mg kg^{-1}). In rest of the diets including wheat grain, wheat bran, maize and mixed diet copper is less. Both Nickel and Lead are sufficiently high in all animal diets except in wheat bran. Wheat bran is low in all the metal content except cobalt which shows that metals are probably taken up and stored in the wheat grain. However all these elements are within the permissible limits of these in fodder except for copper in cotton khal and cobalt in wheat bran. The maximum tolerance of Cd, Co, Cr, Cu, Ni and Pb is 0.5, 0.66, 3000, 10, 50 and 30 mg kg^{-1} for cattle in diet (NRC, 2005).

The level of heavy metals in animal feed of our study are however higher (Tables 4.1.4.5. a-f.& 4.1.4.6) than those reported by Li et al. (2005) in Wisconsin dairy feeds. It was found that mean values of copper were 6.8 mg kg^{-1} in alfaalfa hay, 0.004 mg kg^{-1} in corn,

and 38.2 mg kg^{-1} in corn grain mix. Cr, Cd and Pb were 0.709, 0.074 and 0.198 mg kg^{-1} respectively in alfaalfa hay, 0.519, 0.062, 0.260 mg kg^{-1} respectively in corn silage, 0.326, 0.181, 0.134 mg kg^{-1} respectively in corn grain and 1.829, 0.342 and 0.250 mg kg^{-1} respectively in corn grain mix. Zn and copper were found in high concentration in complete dairy rations due to addition of these in feed supplementation. Chromium, Arsenic, Cadmium and lead were found in low concentration as compared to copper and zinc and none were above the permissible standards. All of these were present in low concentration but present long term risk of bioaccumulation.

Correlation studies between the heavy metal content in milk and the same in diet (Table 4.1.4.8) show that there is a weak positive correlation between the two in some cases. In case of lead the Pearson's correlation is 0.2 in case of cotton khal and mixed diet whereas 0.1 or less in others. In case of nickel it is 0.2 for cotton khal, 0.3 for wheat bran and maize whereas 0.1 or less in others. In case of Copper it is 0.3 for both wheat bran and wheat grain, 0.4 for sugarcane khal and 0.1 or less in others. In case of chromium it is 0.2 for cotton khal and mixed diet and 0.1 for sugarcane khal and maize while for cobalt and cadmium it is 0.1 or less in all types of diets.

Cattle diet induced toxicity of some of the heavy metals in milk has been reported with the world over the last several years. Khattak et al. (2004) reported that the average content of Cd and Cu was 4.1 and 122 mgL^{-1} in cow milk and they revealed the contention that elevated levels of Cd and Cu in milk was directly linked to the diet given to them. They also concluded that cow's feed was prepared from the forages irrigated with industrial and municipal wastewater. Raj et al. (2006) reported that grasses used as a fodder for cattle are found to be high in Cd and Pb than their recommended levels for

cattle diet. They concluded that elevated level of these elements in the milk of grazed cattle is because of grazing on Cd and Pb accumulated grasses.

Apart from diet the other source of heavy metals contamination in milk is drinking water. There are evidences in the literature that elevated level of some of the heavy metals in dairy milk is because of threshold levels of same elements in drinking water. Although the dairy cattle of our study were taking underground water while within the farm but these cattle were taken out for grazing every day where they bath in rivers heavily contaminated with industrial and domestic waste. Water contamination within Peshawar district is reported in the previous literature. Zahoorullah et al. (2003) reported that drinking water of rural areas within Peshawar is heavily contaminated with some of the heavy metals. They concluded that the elevated level of heavy metals in drinking water is because of untreated industrial and municipal effluents in rivers. Qureshi and Khan (2011) studied the heavy metals content in drinking water and milk samples of urban farm within Peshawar and reported that toxicity of Cd, Cr and Pb in the milk of dairy and grazed cattle is because of drinking contaminated water in the urban farm. They showed that Cd, Cr and Pb were above the maximum allowable intake in drinking water of cattle and the heavy metals intake through milk alone was much more than the total daily intake of these heavy metals from all sources. Naz et al. (2011) have also shown in a review study that the water used for irrigation of agricultural fields in Peshawar in particular (elsewhere in general) has elevated levels of various heavy metals, shown below mg L^{-1}.

	Cd	Cr	Cu	Ni	Pb
Industrial effluent (marble,match)	0.056	0.46		0.03	3.20
Domestic sewage	0.26		0.99	0.11	0.75

When heavy metal content was determined in fresh, pasteurized and dry milk samples, the results show that there was greater content of Cr, Cu, Ni and Pb in fresh milk as compared to pasteurized and dry milk whereas Cd and Co was greater in pasteurized and dry milk than in fresh milk. Lopez et al. (1985) reported that heavy metals content in fresh cow's milk was greater than pasteurized milk. In another study Birghila et al. (2008) that Cu, Cr, Ni and Pb was greater in powder milk than fresh and pasteurized milk, whereas Cd content remained less in powder milk than fresh and pasteurized milk. The greater content of Cd and Co in pasteurized and dry milk revealed the contention that during pasteurization contamination may enters.

As discussed above it is concluded that dairy milk is slightly contaminated with these elements. The source of contamination of these elements in milk is not diet alone, as shown by weak positive correlation between the two, but some other sources such as ingesting contaminated soil, grazing over contaminated pastures and drinking wastewater. Therefore in future a complete comprehensive study is required to trace the origin of contamination in milk. No toxicity symptoms were observed in cattle in this study. Heavy metals are more persistent and can remain for decades within cattle body and may result in severe health problem in cattle. Children and adults are more vulnerable to toxic element contamination because of drinking contaminated milk. However milk induced toxicity in children and adults was not studied in this study and needs further investigation.

5.2. Pesticides

The objective of this study was to evaluate the impact of environmental pollution associated with the increase in urbanization and industrialization on dairy milk quality in terms of it's toxic metal and pesticide content and also assess forage induce toxicity of these in milk. Dairy cattle within Peshawar are under constant threat of pesticide contamination because of their persistence and bioaccumulation within their liver and kidneys. Their presence within cattle body causes health risk. The quality and quantity of milk is slowly deteriorating and drinking milk of low quality, indirectly causes health risk to human beings. The main source of these pesticides in dairy cattle milk is diet. The diet given to dairy cattle in dairy farms, either in urban or rural areas, is purchased commercially. The diet is not prepared according to the body requirement of cattle. Therefore, dairy cattle are more vulnerable to risk than grazed cattle. The commonly used diet for cattle is cotton khal, maize khal, sugarcane plants, maize plant and fruit, wheat bran, alfalfa and fresh grass. The plants from which these diets are prepared are sprayed with high doses of organo-phosphate and organo-chloro pesticides. These pesticides are known to produce long term toxic effects in cattle because of their accumulation in the liver and kidneys of cattle. Their persistence within cattle body poses constant threat to them and also to human health. Therefore there was an urgent need to estimate their content in dairy cattle milk in order to provide a baseline for the health authorities to prepare guidelines for dairy farms owners.

When the concentrations of cypermethrin, chloropyrifos and profenofos in the rural and urban milk samples was compared with maximum pesticide residue limits in milk, it was

noted that cypermethrin was 2 to 4 folds greater in all milk samples except for milk sample from rural farm 5. Similarly, chloropyrifos was 4 to 10 folds greater in milk samples from all farms except for urban farms 2 and 3 (Table 4.2.4.1). Profenofos in milk samples of both dairy farms was within the maximum pesticide residue limits in milk (MRL, 2003). The greater content of cypermethrin and chloropyrifos in milk samples revealed the contention that milk is contaminated. Pesticide contamination in dairy milk and products from diet has been reported in the previous literature. The result of this study is in-agreement with the findings of Rothwell et al. (2001). They reported that cypermethrin content was 0.015 mg L^{-1} in the milk samples of dairy cattle and was found to be greater than the maximum allowable limits of cypermethrin in milk. They concluded that the cypermethrin contamination in milk because of their elevated levels in cattle diet. Alvarez et.al (2010), reported cypermethrin contamination in cow milk samples obtained from urban farm. They concluded that this is because of cypermethrin accumulation in body fats of cows. Muir and Baker (1973) reported that the concentrations of organochloro pesticides in cattle milk grazed in orchards were greater than grazed in pastures. They concluded that the difference in the concentrations of pesticides between milk is most probably because of high dose of these pesticides sprayed in the orchards. John et al. (2001) found in Jaipur, India that all milk samples were contaminated with organochlorine pesticides and the levels were more in autumn-winter than in summer. The people in Cartagena, Bogota were exposed to organochlorine compounds through consuming 12.1 mg $g^{-1}day^{-1}$ in pasteurized milk and 100 % of the samples tested were contaminated with the pesticides greater than WHO/FAO limits. (Castilla-Pinedo et al., 2010)

The levels of pesticides in this study were higher than those detected by Bordet (2002) which ranged from 26 to 45 ng gm^{-1} in milk for organophosphorus pesticides and pyrethroids, and also than those found by Pagliuca et al. (2006) who found that 27.40% of the samples were positive in concentrations less than MRLs and 6.89% had contamination with chlorpyrifos ranging from 5 to 18 µg kg^{-1}.

The mean levels of pesticides in raw, dry and pasteurized milk were also compared. Cypermethrin and chlorpyrifos were higher in raw and pasteurized but low in dry milk than the MRLs whereas profenofos was within the permissible limits in all the 3 types of milk. Cypermethrin in this study was detected as 0.17, 0.04, 0.21 mg L^{-1} in pasteurized, dry and raw milk. The level in dry milk was lower but the levels in raw and pasteurized milk were higher than the MRL for cypermethrin in milk of 0.05mg kg^{-1} (MRL, 2003). The level of chlorpyrifos in this study was detected as 0.092, 0.009, 0.092 mg L^{-1} in pasteurized, dry and raw milk respectively whereas the MRL for chlorpyrifos in milk is 0.02mg kg^{-1} (MRL, 2003). This again shows that the level is within the permissible limit for dry milk but high in case of pasteurized and raw milk. The level of profenophos in this study was below the MRL of profenofos in dairy milk. It was detected as 9.8×10^{-4}, 5.7×10^{-4}, 6.8×10^{-3} mg L^{-1} in pasteurized, dry and raw milk respectively which are lower than the MRL for profenofos in dairy milk which is 0.01 mg kg^{-1} (MRL, 2003)

All the three pesticides i.e cypermethrin, chlorpyrifos and profenofos were detected in all types of diet that is commonly given to cattle in our country but the concentration was highest in cypermethrin, lower in chlorpyrifos and lowest in profenofos. However all these values were within the maximum residue limits of these pesticides in animal diet

(MRL, 2003). This shows that diet is not the only source of contamination in milk but there are other sources also like drinking water of cattle which usually contains toxic substances from the industrial, agricultural and domestic waste. Cattle in our country are usually grazed in open fields and drink water from waste water channels. Moreover a comparatively higher level is acceptable in animal diet but a concentration level lower s taken in very minute quantities on a regular basis, they can lead to harmful effects in the long run.

Nonetheless, the levels of cypermethrin and chloropyriphos of this study in cattle diet were less than the levels of same pesticides as reported by Meglar et al. (2010). They found that "residue levels of seven organophosphorus pesticides (OPPs), widely used in crops used for animal feed, were detected between 0.005 and 0.220 mg kg^{-1}." The recovery rate of pesticides in milk samples was between 62.2 - 97.2%. Organophosphorus pesticides were detected in 6.73 % of total milk samples and 8.67 % of raw milk samples. Also Faqir found out that 20-25% milk samples surpassed the MRL levels for chlorpyriphos, cypermethrin and certain other pesticides.

Wong and Lee (1997) conducted a survey in Hong Kong and found that 16.6% of the samples contained organochlorine pesticide residues at levels exceeding the Extraneous Maximum Residue Limits of the Codex Committee on Pesticide Residues. Liaska (1968) found out that a well balanced US diet contains organochlorines 0.02 ppm and organophosphates 0.003 ppm. Most chlorinated insecticides are relatively resistant to processing techniques used for milk and dairy products. Once residues get into milk they are stable and difficult to remove; therefore, the best policy is to prevent their entrance into milk by proper and careful management of the dairy cows.

Chapter 6

CONCLUSION & RECOMMENDATIONS

6.0. CONCLUSIONS & RECOMMENDATIONS

CONCLUSIONS

1. This study was conducted to find out the concentration of heavy metals and pesticides in dairy milk and animal fodder since dairy milk is a commonly consumed food item and the continuous presence of toxic substances in it may have detrimental effects on health.
2. The recovery rates in case of heavy metals were moderate except for Cadmium and Copper which were between ninety to hundred percent.
3. All the metals were found in concentrations slightly greater than the maximum residue limits in dairy milk.
4. Urban farms showed slightly higher concentrations than the rural farms.
5. There was no significant difference between the levels in pasteurized and dry milk but four out of six elements studied were greater in raw milk than the pasteurized and dry milk.
6. All the metals were also detected in animal feed but within the permissible limits of these in fodder except for copper in cotton khal and chromium in sugarcane khal and maize.
7. Five commonly used pesticides were analyzed in milk and fodder. Out of these Cypermethrin levels were higher in all the farms followed by chlorpyriphos whereas the concentration of profenophos was very low in all farms.
8. Urban farm 1 had comparatively higher concentrations of all the pesticides than all the other farms.

9. Raw milk had the highest concentration of all the pesticides whereas pasteurized and dry milk had lower levels but dry milk had the minimum concentrations.
10. The recovery rates of chlorpyrifos were high while that of cypermethrin and profenofos were moderate.
11. The concentrations of cypermethrin and chlorpyrifos were more than the maximum residue limits while that of profenofos were lower. There was not much variation in the levels of cypermethrin and chlorpyrifos in all the farms.
12. All the pesticides were also detected in animal feed but within the permissible limits of these in fodder.
13. There was a weak positive correlation between the metals level in milk and animal fodder probably because of differential absorption/selective bioaccumulation in liver and kidneys..
14. Whereas the weak relationship between pesticide level in milk and animal diet is probably due to their differential degradation and differential elimination from the body.
15. The results show that heavy metals and pesticides gain entry into the dairy milk probably through grazing in open pastures rather than taking the commercially available prepared feed because the concentrations in feed were within the permissible levels.
16. The cattle feed which is prepared scientifically is irrigated with clean water and cut at a time which is more than the elimination half life of the pesticides.

Cattle get contaminated feed by grazing in open pastures sprayed with pesticides without the elimination half life being lapsed.

17. The toxic heavy metals may gain entry into the body of cattle by grazing in open pastures irrigated with drainage channels containing toxic metals from industrial and municipal waste and also drinking waste water from drainage channels.
18. Since heavy metals and pesticides are stored in adipose tissue of body and show bioaccumulation therefore even when taken in small amounts regularly they can produce long term toxic effects on human body.

RECOMMENDATIONS

1. Although heavy metals and pesticides can be removed from any liquid medium by complex techniques of chelation and treatment with zero-valent ions like iron and zinc but this is simply impracticable for a daily consumable item like water or milk
2. he best is to prevent their entry into the medium by simple inexpensive method of good agricultural practices GAP) which means no use of fertilizers but instead fertilizing the soil by use of natural manure, leaving some of the wheat and maize straw /husk, sugar cane bagasse and other crop residues in the field to fertilize the soil or alternate farming of cash crops with legumes etc which provide nitrogen & help in fertilization,.

3. Infrastucture should be improved and inexpensive analytical services should be provided to the farmers for determining the concentration of various toxicants in animal fodder and crops.
4. General education of the farmers in the form of free refresher courses, with an incentive, should be conducted to educate them about the harmful effects of grazing in open fields and drinking in contaminated drainage channels.
5. They should also be informed about the general hygiene of the cattle and the dangerous effects of using injections in the cattle.
6. But at the same time the dairy farmers should be facilitated by providing clean animal diet at low rates and easily accessible outlets.
7. They can also be provided soft loans to construct scientific and hygienic farms with the provisions of clean drinking water.
8. Livestock inspectors should check them periodically and can reward or fine them accordingly.

REFERENCES

REFERENCES

Abdallah, M.I.M., 2005. Evaluation of some Heavy Metals Residence in whole milk powder used at confectionary plants regarding their Public Health Significance. Research of Food Inspection Lab, Damietta sea Port. Animal Health Research Institute. Agricultural Research Center, Giza, Egypt.

Agnihorti, N.P., Jain, H.K. and Gajbhiye, V. T., 1986. Persistence of some synthetic pyrethroid insecticides in soil, water and sediment- part I. Entomology Research 10(2): 147-151.

Alloway, M., 2005. Heavy metals in soils. Essentials of medical geology: impacts of the natural environment on public health. Academic Press, 812 pp.

Alvarez, M.F., Lamas, J.P., Chao, M.C., Jares, C.G., Llompart, M., Lores, M and Dagnac, T., 2010. Multiresidue Determination Of Pesticides In Edible Animal Tissues By Novel Solid Phase Microextraction And Chromatographic Techniques. Environmental Monitoring 12 (10): 1864-1875.

Anastassiades, M.S., Lehotay, D., Stejnbaher and Schenck, F., 2003. Fast and easy multi residue method employing acetonitrile extraction/partitioning and dispersive solid phase extraction' for the determination of pesticide residues in produce. AOAC International 86 (2): 412-431.

Anastasio, A., Caggiano, R., Macchiato, M., Cattellani, P., Ragosta, M., Paino and Cortessi, M.L., 2006. Heavy Metal Concentration in Dairy products from sheep milk collected in two regions of Southern Italy. Acta Veterinaria Scandinavica 4 (7): 69-74.

AOAC, 1989. Official methods of Analysis of the Association of Official Analytical Chemists, 14th edition, Williams, S.

AOAC. 1990. Official methods of analysis of the Association of Official Analytical Chemists. 15th edition. Washington, DC, Association of Official Analytical Chemists.

Asami, M.O., 1984. Pollution of soils by cadmium in Changing Metal Cycles and Human Health, ed. Nriagu, J. O., 95-111 (Springer Verlag Berlin).

Ashton, W.M. and Yousef, I.M., 1966. A study of the composition of Clun Forest ewe's milk. II. Mineral constituents. Agricultural Science 67: 77-80.

Aslam, B., Javed, I., Khan, F.H. and Rehman, Z.U., 2011. Update of heavy metals from sewage sludge in the milk of Goat and Cattle during summer season. Pakistan Veterinary Journal, 31: 75-77.

ATSDR, 1989. Agency For Toxic Substances and Disease Registry, Public Health Statement. Cadmium. ATSDR/ TP-88/02

ATSDR, 1997. Toxicological profile for Nickel (update). U.S. Department of Health & Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, U.S. Government Printing Office.

Auza, N. J., Olson, N. W., Murphy, M. J. and Linn, J. G., 1999. Diagnosis and treatment of copper toxicosis in ruminants. American Veterinary Medical Association 214:1624-1628.

Awan, H.N., Tahir, H.A. and Rafique, U., 2005. Determination of zinc and lead in raw and processed milk. Rawal Medical Journal, 30 (2): 76-78.

Bacci, E., Calamari, D., Gaggi, C. and Vighi, M., 1987. An approach for the prediction of environmental distribution and fate of cypermethrin. Chemosphere., 16(7): 1373-1380.

Bahatia, I. and Choudri, G.N., 1996. Lead poisoning of milk. The basic need for the foundation of human civilization. Indian Journal of Public Health, 40: 24-26.

Barnes, J., Anderson, L. and Phillipson,D., 2002. Herbal Medicine, 2nd Edition. Pharmaceutical Press, London.

Barry, P.S., 1975. Lead concentration in human tissues. Britain Journal of Industrial Medicine 32:119-39.

Battu, R.S., Singh, B. and Kang, B.K., 2004. Contamination of liquid milk and butter with pesticide residues in the Ludhiana district of Punjab state, India. Ecotoxicology Environment Safety 59 (3): 324-31.

Becker, D. E. and Smith, S.E., 1951. The level of Cobalt tolerance in Sheep. Animal Science, 10: 266-271.

Besong, S., Jackson, J., Trammell, S. and Amaral-Phillipa, D., 1996. Effect of supplemental chromium picolinase on liver triglycerides, blood metabolites, milk yield, and milk composition in early lactation cows. Dairy Science 79: (Suppl-1), 97.

Birghila, S., Dobrinas, G. and Stanciu, A., 2008. Determination of major and minor elements in milk through ICP-AES. Environment Engineering Management 7: 805-808.

Bissacot, D.Z. and Vassilieff, I., 1997. Pyrethroid residues in milk and blood of dairy cows following single topical applications. Veterinary Human Toxicology 39 (1): 6-8.

Blakley, B. R., 1984. A retrospective study of lead poisoning in cattle. Veterinary Human Toxicology, 26; 505.

Blanco-Penedo, Shore, R. F., Miranda, M., Benedito, J. L., and López-Alonso M., 2009. Factors affecting trace elements status in calves in NW Spain. Livestock Science 123(3): 198-208.

Blokhima, R. I., 1970. Cobalt and goiter, in Trace Element Metabolism in Animals, ed. Mills, C. F. 426-32 (livingstone, Edinburgh).

Blunden, S. and Wallance, T., 2003. Tin in canned food: a review and understanding of occurrence and effect. Food and Chemical Toxicology, 41: 1651-1662.

Bolygó, E and Hadfield,S.T., 1990. An on-column capillary gas chromatographic method for the analysis of synthetic pyrethroid residues on fish eggs. High Resolution Chromatography 13(7): 457–460.

Bordet, F., Inthavong, D. and Fremy, J.M., 2002. Interlaboratory study of a multiresidue gas chromatographic method for determination of organochlorine and pyrethroid pesticides and polychlorobiphenyls in milk, fish, eggs, and beef fat. AOAC International 85 (6): 1398-1409.

Boyne, R. and Arthur, J. R., 1986. Effects of molybdenum or iron induced copper deficiency on the viability and function of neutrophils from cattle. Research Veterinary Science 41: 417-19.

Bowen, H.J.M., 1979. Environmental Chemistry of the Elements, Academic Press, New York.

Bradley, C. H., 1993. Copper poisoning in a dairy herd fed a mineral supplement. Canadian Veterinary Journal 34(5):287-292.

Bradshaw, A.D., McNeilly, T. and Gregory, R.P.G., 1965. Industrialization, evolution and the development of heavy metal tolerance in plants, in Ecology and Industrial Society, ed. Goodman, G.T., Edwards, R. W. and Lambart, J.M., Br. Ecol. Soc. Symp., 5, 327-34.

Brown, D. 2005. The Determination of Multiple Pesticide Residues in Fruits And Vegetables using Triple Quad GC/MS/MS. Boycote Laws-Birmingham UK.

Brown, S.S. and Sunderman, F. W., Jr., 1980. Nickel Toxicology. Academic Press London.

Browning, E., 1969. Toxicity of Industrial Metals (Butterworths, London).

Bruce, A. A. and Richard, W. M., 1987. Weeds: and illustrated botanical guide to the weeds of Australia. Science, 255p.

Buckingham, S. D., Lapied, B., Corronc, H. L., Grolleau, F. and Sattelle, D. B., 1997. Imidacloprid Actions on Insect Neuronal Acetylcholine Receptors. Experimental Biology 200: 2685-2692.

Bunker,V.W., Lawson, M. S. and Delves, H. T., 1984. The uptake and excretion of Chromium by the elderly. American Journal Clinical Nutrition 39: 797-802.

Burrows, G. E., 1981. Lead toxicosis in domestic animals: a review of the role of lead mining and primary led smelter in the United States. Veterinary Human Toxicology 23: 337-343.

Burrows, D., 1983. Chromium: Metabolism and Toxicity, Preface (CRC Press, Boca Raton, Florida).

Burton, J. I., 1995. Supplemental chromium; Its benefits to the bovine immune system. Animal Feed Science Technology, 53; 117.

Burton, J. L., Mallard, B. A. and Mowat, D. A., 1993. Effect of supplemental chromium on immune responses of periparturient and early lactation dairy cows. Animal Sciences 71; 1532.

Burton, J. L., Mallard, B. A. and Mowat, D. A., 1994. Effects of supplemental chromium on anitobody responses of newly weaned feedlot calves to immunization with infectious bovine rhinotracheitis and parainfluenza 3 virus. Canadian Journal of Vetinary Research 58; 148.

Cai, O., Long, M.L., Zhu,M., Zhou,Q.Z., Zhang, L. and Liu, J., 2009. Food chain transfer of cadmium and lead to cattle in a lead–zinc smelter in Guizhou, China. Environmental Pollution; 157 (11): 3078-3082.

Caggiano, R.S., Sabiaa, S., Emiliob, M., Macchiatob, M., Anastasioc, A., Ragostad, M. and Piano,S., 2004. Metal levels in fodder, milk, dairy products and tissues sampled in bovine farms of Southern Italy. Environmental Research, 99: 48-57.

Cardeal, Z.L. and Dias, P.D.M., 2006. Analysis of Organophosphorus pesticides in whole milk by solid phase micro extraction gas chromatography method. Environmental Science Health B 41 (4): 369-75.

Carlile, W. R., 2006. Pesticide Selectivity, Health and The Environment. Cambridge University Press, 310p.

Carson, R. 1962. Silent Spring. New York: Houghton Mifflin Company.

Castilla-Pinedo, Y., Alvis, E.L and Alvis-Guzmán,N., 2010. Estimating exposure to organochlorine compounds from consuming pasteurised milk in Cartagena, Colombia. Review Salud Publication (Bogota), 12 (1): 14-26.

Chapman, R.A., Tu, C.M., Harris, C.R. and Cole, C., 1981. Persistence of five pyrethroid insecticides in sterile and natural, mineral and organic soil. Bulletin Environment Contamination Toxicology 26: 513-519.

Chen, T.H., Henderson-Sellers, A., Milly, P.C.D., Pitman, A.J., Beljaars, A.C.M., Polcher, J., Abramopoulos, F., Boone,A., Chang,S., Chen, F., Dai, Y., Desborough, C.E., Dickinson, R.E., Dümenil, L., Ek, M., Garratt, J.R., Gedney, N., Gusev, Y.M., Kim, J., Koster, R., Kowalczyk, E.A., Laval, K., Lean, J., Lettenmaier, D., Liang, X., Mahfouf, J.F., Mengelkamp, H.T., Mitchell, K., Nasonova, O.N., Noilhan, J., Robock, A., Rosenzweig, C., Schaake, J., Schlosser, C.A., Schulz, J.-P., Shao, Y., Shmakin, A.B., Verseghy, D.L., Wetzel, P., Wood, E.F., Xue, Y.,Yang, Z.-L. and Zeng, Q.,1997. Cabauw experimental results from the Project for Intercomparison of Land-Surface Parameterization Schemes.

Climate 10, 1194-1215.
Ciscato, C.H., Gebara, A.B. and Spinosa, H.S., 2002. Pesticide residues in cow milk consumed in São Paulo City (Brazil). Environmental Science and Health B, 37(4): 323-330.
CISRO., 2007. Nutrient requirement of domestic animals. CISRO publications, Oxford Street, Collingwood VIC, Australia.
Code of Federal Regulations, 2010. CODEX online Pesticide details for Cypermethrin. FAO/WHO 2010.
Collins, R. A., Harper, A. E., Schreiber, M., and Elvehiem, C. A., 1951. The folic acid and vitamins B12 content of the milk of various species. Journal of Nutrition 43:313.
Cong, X., Xue, N., Wang, S., Li, K. and Li, F., 2010. Reductive dechlotination of organochlorine pesticides in soils from an abandoned manufacturing facility by zero-valent iron. Science of Total Environment, 408 (16): 3418-3423.
Connel, D.W. and Miller, G.T., 1984. Chemistry of ecotoxicology pollution. John Wiely and Sons, New York.
Coulston, F., Goldberg, L. and Griffin, T., 1972. Safety evaluation of Dowco 179 in humans. Inst. Exp. Pathol. Toxicol., Albany Medical College.
Cox, L., Koskinen, W. C. and Yen, P. Y.M., 1997. Sorption-desorption of imidacloprid and its metabolites in soil. Agriculture Food Chemistry 45: 1468-1472.
Cruz, G.C., Din, Z., Feri,C.D., Balaoing,A.M., Gonzales,E.M., Navidad,H.M., Schlaaff, M.F. and Winter, J., 2009. Analysis of toxic heavy metals (Arsenic, Lead and Mercury) in selected infant formula milk commercially available in the Philippines by AAS. International Scientific Research Journal, 1 (1): 40-51.
Cunningum, W. P. and Saigo, B. W., 1997. Environmental Science, a global concern 4thedition. p. 389. WMC Brown Publisher New York.
Daniel, B. and Edward, A.K. 1995. Environmental science. Earth as a living planet, pp 278-279. John Wiley and sons, Inc. New York.
Debashis, R., Bharathidhasan, S., Veena, M., Harjit, K. and Neelam, K., 2009. Heavy Metal Contents in Cow and Buffalo Milk Samples from Haryana. Indian Journal of Animal Nutrition, 26 (1): 29-33.
Dey, S., Dwivedi, S. K. and Swarup, D., 1996. Lead concentration in blood, milk and feed of lactating buffaloes after acute poisoning. Vertinary Research, 138: 336.
Dobrznski, Z., Gorecka, H., Kolacz, R. and Gorecki, H. J., 1994. Effect of pollution from copper industry on heavy metal concentration in green forage, blood serum of dairy cattle and in milk. Proceedings of the 8th International Congress on Animal Hygiene. Sept 12-16, St, Paul Minnesota, USA., pp; 29-32.
Dobrznski, Z., Kolacz, R., Garecka, H., Chojnacka, K. and Bartkowiak, A., 2005. The contents of microelements and trace elements in Raw milk of cows in the Silesian Region. Polish Journal of Environmental Studies14 (5): 685-689.
Donaldson, R. M. and Barreras, R. F., 1966. Intestinal absorption of trace quantities of chromium. Laboratory Clinical Medicine 68: 484-9.
Dowding, M.F., 1978. Lead. Met. Materials (UK), July, 2-36.
Duff, M. and Voglino, M., 2010. Rapid and Robust Multi-Residue Pesticides Analysis Using the Varian 320-MS GC Triple Quadrupole Mass Spectrometer. Multiresidue Analysis by Quechers Method.
Duffus, J.H., 1983. Environmental Toxicology. Edward Arnold Publishers Limited, London. Pg.220.

Dwivedi, S. K., Swarup, D., Dey, S. and Patra, R.C., 2001. Lead poisoning in cattle and buffalo near primary lead-zinc smelter in India. Veterinary Human Toxicology, 43:93-94.
Earley, B., Fallon, R. J., Murray, M. and Farrell, J. A., 2002. Immunological and Haematological responses in Calves supplemented with organic chromium and offered different calf milk replacers. Irish Journal of Agricultural and Food Research, 41 (1): 87-93.
Economic Survey of Pakistan, 1999-2000 & 2010-11. Annual reports of economic survey of Pakistan. Bureau of Statistics Govt of Pakistan.
Edbon, L., 2001. Trace element speciation for environment, food and health. Royal Society of Chemistry (Great Britain). 391p.
Ellen, G., Van Den Bosch-Tibbesma and Douma, F. F., 1987. Nickel content of various Dutch foodstuffs. Z Lebensm Unter Researchers, 179: 145-156.
Elsayed, E.M., Hamed, A.H., Badran,S.M. and Mostafa, A.A., 2011. A survey of Selected Essential and Toxic Metals in Milk in Different Regions of Egypt using ICP-AES. International Journal of Dairy Sciences, 6: 158-164.
Elzerman, A.W. and Paddock, R.W. 2000. Environmental Chemistry of water 2000 and beyond. Symposia papers. Presented before the division of Environmental Chemistry, American Chemical Society, San Francisco, CA . Vol. 40; No.1: p. 428-
Enb, A., Abou Donia, M.A., Abd Rabou, N.S., Abou Arab, A.A.K. and El Senaity, M.H. 2009. Chemical composition of raw milk and heavy metal behaviour during processing ofmilk products. Global Veterinaria, 3 (3): 268-275.
Endrews, E. D. and Hart, L. I., 1962. A comparison of vitamin B12 concentrations in livers and kidneys of cobalt-treated and mildly cobalt deficient lambs. New Zealand Journal of Agricultural Research, 5: 403-408.
EPA, 2000. Standards for the use and disposal of biosolids EPS/832/F-00-064, Office of Water, Washington, DC.
Environmental Fate of pesticides, Ministry of Agriculture. www.agf.gov.bc.ca/pesticides/c 2.htm
European Union legislation 2006. Cotaminants in food. CBI.
European Union, 2002. Heavy metals in wastes. Final Report.
EUCR, 2010. Annual report on food contamination.
Fang, Yun-Zhong, Yang, S., Wu Guoyao., 2002. Free radicals, antioxidants and nutrition. Nutrition, 18: 872-879.
FAQs on Pesticides in Milk, 2008. Developed by: The Organic Center.USA
Fitzgerald, P.R., Peterson, J. and Lue- Hing,1985. Heavy metals in tissues of cattle exposed to sludge treated pastures for eight years. American Journal of Veterinary Research, 46: 703-7.
Flanjak, J., and Lee, H. Y., 1979. Trace metal content of livers and kidneys of cattle. Science of Food and Agriculture, 30 (5) : 503-507.
Florea, T., Huszthi, S.O. and Costin., G.M., 2006. Heavy metal contamination in milk and cheese. The annals of the University of Dunarea de Jos of Galati. Fascicle VI-Food technology.
Flores-Cespedes, F., Gonzales-Pradas, E., Fernandez-Perez, M., Villafranca-Sanchez, M., Socias-Viciana M. and Urena-Amate, M. D., 2002. Effects of Dissolved Organic Carbon on Sorption and Mobility of Imidacloprid in Soil. Environmental Quality, 31: 880-888.
Friberg,L., Elinder,C., Gand, K. and Jelstrom,T., 1992. IPCS Environmental Health Criteria 134. Cadmium. World Health Organization, Geneva.

Freire, A. C. T., Gontijo, R. M., Pessoa, J. M., Souza, R., 1981. Effect of xylazine on the electrocardiogram of sheep. Britain Veterinary Journal, 137: 590-595.
Frobish, R. A. and Davis, C. L., 1977. Theory involving propionate and vitamin B~2 in the low milk fat syndrome. Dairy Science, 60: 268-273.
Fossin, M., 2006. Environmental Fate of Imidacloprid. Environmental Monitoring Department of Pesticide Regulation 1001 I Street Sacramento, CA 95812–4015.
Fox, D.A., Campbell, M.L. and Blocker, Y.S., 1997. Functional alterations and apoptotic cell death in the retina following developmental or adult lead exposure. Neurotoxicology, 18: 645-664.
Gazzotti, T., Sticca, P., Zironi, E., Lugoboni, B., Serraino, A. and Pagliuca, G., 2009. Determination of 15 organophosphorus pesticides in Italian raw milk.Bulleten Environmental Contamination Toxicology, 82 (2): 251-254.
Gilani,S.T.S., Ageen, M., Shah, H. and Raza, S., 2010. Chlorpyrifos Degradation In Soil And Its Effect On Soil Microorganisms. Animal and Plant Sciences, 20(2): 99-102.
Golfinopoulus, S. K., Nikolaou, A. D., Kostopoulou, M. N., Xilourgidis, N. K., Vagi, M. C. and Lekkas, D. T., 2003. Organochlorine pesticides in the surface waters of Northern, Greece, Chemosphere, 50: 507–516.
Gross, S.B., Yeagar, D. W. and Middendorf, M.S., 1976. Cadmium in liver, kidney and hair of humans, foetal through old age. Toxicological Environmental Health 2: 153-167.
Gutenmann, W.H., St. John, Jr., L.E. and Lisk, D.L., 1968. Metabolic studies with O,O- diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate (Dursban) insecticide in a lactating cow. Agriculture Food Chemistry, 16(1): 45-47.
Hackbart, K.S., Ferreira, R. M., Dietsche, A. A., Sochat, M. T., Shaver, R. D., Wiltbank, M. C., and Fricke, P. M., 2010. Effect of dietary organic zinc, manganese, copper and cobalt supplementation on milk production, follicular growth, embryo quality and tissue mineral concentrations in dairy cows. Animal Sciences, 88 (12): 3856-3870.
Hall, J. S., Leahey, J.P. and Curl, E. A., 1981. Cypermethrin: Photodegradation on a soil surface. ICI Plant Protection Division. Data Package Report No. RJ 0192B. DPR No. 418-056.
Hamrick, I. and Counts, S. H., 2008. Vitamin and mineral supplements. Wellness and Prevention, 35:729-747.
Hammer, D. J., Finklea, J. F. and Creason, J. P., 1971. Cadmium exposure and human health effects in Trace Substances in Environmental Health, ed. Hemphill, D.D., 269-87. (uni. Missouri Press Columbia, Mo,).
Harding, F., 1995. Milk quality. Blackie Academic and Professional, London.
Harris, E. D., 1997. Copper. In: O'Dell, B.L. and. R.A. Sunde, (Eds.), Handbook of Nutritional. Essential Mineral Elements. Marcel, New York, pp: 231-273.
Heffron, C. L., Reid, T. J., Elfving, C. D., Stoewsand, G. S., Haschek, W. M., John, N., Telford, A., Keith, F., Thomas, F., Parkinson, C. and Bache, A., 1980. Cadmium and zinc in growing sheep fed silage corn grown on municipal sludge-amended soil. Agriculture Food and Chemistry, 28 (1): 58-61.
Hill, C. H., 1976. Mineral Interrelationships in Trace Elements in Human Health and Disease, ed. Prasad, A.S., Vol. 2, p 281-298. Academic Press, New York.
Hooda, P., 2010. Trace elements in soils. John Wiley and Sons, Science, 616 p.
Hosnedlova, B., Travnicek, J., and Chastny,V., 2005. Zinc and copper concentration in milk of dairy cows in South Bohemian region. ISAH, 1: 256-259.

Hovda, L. R. and Hooser, S. B. Toxicology of newer pesticides for use in dogs and cats. Veterinary Clinic Small Animal, 32: 455-467.

Howell, J. M., 1996. Toxicities and excessive intakes of minerals. In: Detection and treatments of mineral nutrition problems in grazing sheep. ACIAR Monograph No. 37. (D. G. Master and C. L. White. eds) pp 95-117. Watson Ferguson and Cooperation, Brisbane.

Horwitz, W., 1982. Evaluation of analytical methods used for regulation of foods and drugs. Analytical Chemistry, 54: 67-76.

Hu X. Y., He J. Z., Gao Y. Z., 2002. Soil remediation in mining and control to acid water. Protect Measure Soil Environment, 3: 135-140.

Ifflaender, U., Mucke, W. and Esser, H.O., 1974. Distribution, degradation and excretion of CGA 15'324 in the rat. Project No.: 16/74. Unpublished report from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Ciba-Geigy Ltd, Basle, Switzerland.

Ilyas, M., 2003. Community Medicine and Public Health. Chap.Medical Entomology. p. 1091. 6th Edition.Time Publisher, Medical Division, Karachi, Pakistan

Imadullah, M., Khan, N., Khan, M. J., Khattak, R. A., 2001. Heavy metals (Cu, Fe, Mn, Zn, Cd, Co, Ni, Mo, Cr and Pb) status in some selected soils of Peshawar and Nowshera districts. Journal of Chemical Society of Pakistan, 23(1): 23-30.

Imidacloprid Technical Fact sheet; 2010. National Pesticide Information Centre (NPIC) http://npic.orst.edu/factsheets/imidacloprid.pdf.

INCHEM. 2001. Toxicological Evalua_ ons: Imidacloprid. Interna_ onal Program on Chemical Safety(IPCS).

Integrated Risk Information System (IRIS)., 1998. Copper ((CASRN 7440-50-8) US Environmental Protection Agency.

IPCS, 1992. Environmental Health Criteria 123; Alpha- and Beta Hexachlorocyclohexanes, Geneva, World Health Organization, 170 p.

Ivie, G. W. and Dorough, H. W., 1977. Fate of pesticides in large animals. Academic Press Inc. UK.

Jan, F.A., Ishaq, M., Khan, S., Shakirullah, M., Asim, S.M. and Ahmad, I., 2011. A comparative study of bioaccumulation of some essential and toxic metals in human's blood through different food chains in industrially contaminated area and relatively less polluted area. Environmental Sciences, 23 (10):

JECFA:33. Report: 1989.Evaluation of certain food additives and contaminants. WHO Technical Report Series 776. Genf. T6 '10JECFA:33.Report: 1993.Evaluation of certain food additives and contaminants. WHO Technical Report Series 837.

Jin, H. and Webster, G.R.B., 1998. Persistence, penetration and surface availability of cypermethrin and its major degradation products in elm bark. Agriculture Food Chemistry, 46: 2851-2857.

JMPR 2007. www.fao.org/ag/AGPP/Pesticid/JMPR/Download/2007_rep/Profenofos.pdf.

John, P.J., Bakore, N. and Bhatnagar, P., 2001. Assessment of Organochlorine pesticide levels in dairy milk and buffalo milk from Jaipur city, Rajasthan India. Environment International, 26,(4): 231-236.

Josland, S. W., 1937. The effect of feeding excess cobalt to healthy sheep. New Zealand. Science and Technology, 19: 31-37.

Judson, G. J., Shallow, M., and Ellis, N. J. S., 1997. Vitamin B_{12} responses to cobalt pellets in beef cows. Australian Veterinary Journal 75: 660-662.

Karabasanavar, N. S., Singh, S. P., Singh, M. K., 2012. Monitoring for chloropyrifos residues in animal feed and fodder of Tarai region of Uttarakhand, India. Toxicology and Environmental Chemistry, 94 (2): 275-280.

Karpel, J.T. and Peden, W. H., 1972. Copper deficiency in long term parenteral nutrition. Pediatrics, 80: 32-36.

Kaufman, D.D., Russell, B.A., Helling, C. S. and Kayser, A. J., 1981. Movement of cypermethrin, decamethrin, permethrin and their degradation products in soil. Agriculture and Food Chemistry. American Chemical Society, Washington D.C. 239-245.

Kazi, T.G., Jaibani, N., Baig, J.A., Afridi, H.I., Khandro, G.A., Arain, M.B., Jamali, M.K. and Shah, A.Q., 2009. Determination of toxic elements in Infant Formulae by using electro thermal atomic absorption spectrometer. Food and chemical toxicology, 47: 1425-1429.

Kegley, E. W., and Spears, J. W., 1995. Immune response, glucose metabolism, and performance of stressed feeder calves fed organic and inorganic chromium. Animal Sciences, 73: 2721.

Kehoe, R. A., 1961. The metabolism of lead in man in health and disease. Journal of the Royal Institute of Public Health and Hygiene 24, 81, 101, 129, 177.

Kennish, M.J. 1992. Ecology of Estuarines Anthropogenic Effects. CRC Pregs. Boca Raton. 494p.

Khattak, R,A., Haq, M.U., Puno, H.K. and Saif, M.S., 2004. Forages induced dairy metal contamination. Journal of Chemical Society of Pakistan, 26 (3): 279-284.

Kidd, H. and James, D.R., 1991. The Agrochemicals Handbook, 3rd Edition. Royal Society of Chemistry Information Services. Cambridge, UK.

Kincad, R. L. and Schoa, M., 2007. Effect of cobalt supplementation during late lactation and early lactation on milk and serum measures. Dairy Science, 90: 1880-1888.

Kinsara, A.A. and Farid, S.M., 2006. Concentration of Trace Elements in Human and Animal Milk in Jeddah, Saudi Arabia. Medical Journal of Islamic World Academy of Science, 16 (4): 181-188.

Klevay, L.M., 1975. Ratio of zinc to copper in diets in the United States. Nutrition Reports International, 11: 237-242.

Koh, T. S., and Judson, G. J., 1986. Trace elements in sheep grazing near a lead-zinc smelting complex at Port Pirie, South Australia. Bulletin of Environmental Contamination and Toxicology, 37 (1): 87-95.

Kollman, W. and Segawa R., 1995. Interim Report of the Pesticide Chemistry Database. Environmental Hazards Assessment Program. Department of Pesticide Reguls.

Korkeala, H., Sofback, S. and Hirn, J., 1984. Effect of cadmium on the growth of Lactobacillus lactis, L. helveticus and Streptococcus thermophilus in milk. Dairy Research, 51 (4): 591-596.

Krieger, N., 2001 Embodiment a conceptual glossary for epidemioligy. Epidemiol Community Health, 55: 693-700.

Krumwiede, D., Sagitate, K. and Joachim, H., 2008. Multiresidue low level quantification of pesticides in food using highly resolved selected reaction monitoring on a triple quadrupole GC/MS System. Thermo Fisher Scientific Bermen Germany, Thermo Fisher Scientific Yokohama Japan.

Kwarta Jr., Hemm, R. F., Pollock, J. J., Christian, M. S., Usui, T., Suzuki, M., and Yago, Nagataka, 1986. Common Health, 34:165-169.

Label Review Manual, 2009. U.S Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs.

Lagaly, G., 2001. Pesticide clay interactions and formulations. Applied Clay Science, 18 (1-5): 205-340

Lante, A.G., Lomolino, M., Cagnin and Spettoli, P., 2004. Content and characterization of minerals in milk and in crescenza and squacquerone Italian fresh cheese by ICP-OES. Food Control, 17: 229-233.

Lau, J., Meng, C.K. and Gushue, J., 2008. Analyzing Compounds of Environmental Interest Using an LC/Q-TOF Part 3: Imidacloprid and Manool. Agilent Technology Publication5989-9129 EN.

Lauri, H.J., Lajunen, P. and Perämäki., 2004. Spectrochemical analysis by atomic absorption and emission. Royal Society of Chemistry, 342 p.

Leader, H., and Casida, J. E., 1982. Resolution and biological activity of the chiral. Agriculture Food Chemistry 30: 546.

LeBlanc, S., 2006. Monitoring programs for transition dairy cows. World Buiatrics Congress, Nice-France.

Leahey, J. P., 1985. Metabolism and environmental degradation, in the Pyrethroid Insecticides. Taylor and Francis, London. 263-342.

Lewinsky, A.A., 2007. Hazardous materials and wastewater: treatment, removal and analysis. Nova Science Publishers, New York.

Levine, R. A., David, H. P., Streeten, and Doisy, R. J., 1968. Effects of oral chromium supplementation on the glucose tolerance of elderly human subjects. Metabolism 17, (2):114-125.

Livestock Census, 1996. Agriculture Census Organization, Statistic Division, Govt of Pakistan, Gulberg, Lahore.

Li, Y., McCwry, D.F., Powel, J.M., Saam, H. and Jackson, S.D. 2005. A Survey of selected heavy metals concentrations in Wisconsin Dairy Feeds. Dairy Science American Dairy Science Association, 88: 2911-2922.

Li, Y.B. and Zhao X.H., 2010. Preliminary study on the degradation of seven organophosphorus pesticides in bovine milk during lactic acid fermentation or heat treatment. African Journal of Microbiology Research, 4 (11): 1171-1179.

Liaska, B.J., 1968. Effects of Processing on Pesticide Residues in Milk. Animal Science, 27: 827-830.

Licata, P., Trombetta, D., Cristani, M., Giofre, F., Martino, D., Calo, M. and Niccari F., 2004. Levels of toxic and essential metals in samples of bovine milk from various dairy farms in Calabria Italy. Environmental International, 30 (1): 1-6.

Lindsay, D.G.,1986. Estimation of dietary intake of chemicals in food. Food Additive Contamination, 3:71-88.

Longhurst, R. D., Roberts, A.H.C. and Waller, J. E., 2004. Concentrations of arsenic, cadmium, copper, lead and zinc in New Zealand pastoral top soils and herbages. New Zealand Journal of Agricultural Research, 47: 23-32.

Loosli, R., 1985. Project No.: 850910. Unpublished summary report from Ciba-Geigy Ltd., Basle, Switzerland. Submitted to WHO by Ciba- Geigy Ltd., Basle, Switzerland.

Lopez, A., Collins,W.F. and Williams, H.F., 1985. Essential Elements, Cadmium, and Lead in Raw and Pasteurized Cow and Goat Milk. Dairy Science, 68 (8): 1878-1886.

Mackenzie, E. J. and Purves, D., 1975. Toxic metals in sewage sludge. Chem. Ind., 4th January, 12-13.

Marston, H. R., 1970. The requirements of sheep for cobalt or for vitamin B_{12}. Britain Journal of Nutrition, 24: 615-633

Material Safety Data Sheet. 2010. Manufacturer informationservice. Rayfull Holding Co.,Ltd. 16 floor 2a. Daziran City Light Building Jinxiu Rd Wenzhou Zhejiang China.

Matsuda, K. and Sattelle, D. B., 2005. Mechanism of Selective Actions of Neonicotinoids on Insect Acetylcholine Receptors. New Discoveries in Agrochemicals: American Chemical Society Symposium Series; Clark, J. M.; Ohkawa, H., III, Eds.; Oxford University Press: Oxford, UK. 172-183p.

Maxcy, K. F. and Rosenau, M. J., 1984. Public Health and Preventive Medicine. Medically Impotant Arthropods and their Control. Pg.521. 11th edition. Appleton Century Crofts. 292 Madison Avenue, New York, 10017.A publishing division of Prentice Hall, Inc.

McDowell, L. R., 2003. Minerals in animal and human nutrition, 2nd edition., Elsevier Health Sciences 660p.

McDowell, L. R., 1996. Feeding minerals to cattle on pasture. Animal Feed Science Technology, 60: 247-271.

McDowell, L. R., 1997. Minerals for grazing ruminants in tropical regions, 3rd Edition, University of Florida, Gainesville, Fla.

McDowell, R. L., 1987. World review. Animal Products Journal, 23:19.

McDowell, J., 2010. Encyclopedia of human body systems. Science, 667p.

Melgar, M.J., Santaeufemia, M. and Garcia, M.A., 2010. Organo phosphorus pesticide residues in raw milk and infant formulas from Spanish Northwest. Environmental Science Health B, 45(7): 595-600.

Mertz, W., 1993. Chromium in human nutrition. A review. Nutrition, 123: 626-633.

Milchunas, D. G., Lauenroth, W. K. and Bruke, I. C., 1998. Livestock grazing: animal and plant biodiversity of shortgrass steppe and the relationship to ecosystem function. Oikos, 83 (1): 65-74.

Miles, Inc. 1993. NTN 33893 (imidacloprid) - Environmental fate summary. Volume No. 51950–0113. Department of Pesticide Regulation, Sacramento, CA.

Miller, G.J., Varnell, T.R. and Rice, R.W., 1967. Fatty acid composition of certain oven tissues as affected by maintenance level rations of roughages and concentrates. Animal Science, 26: 41-45.

Miller, W. J., 1979. Dairy cattle feeding and nutrition. Academic Press, 411 p.

Ministry of Agriculture, Fishries and Food, 1982. 8th Report of the Steering Group on Food Surveillence; the Working Party on the Monitoring of Food Stuff for Heavy Metals (HMSO, London).

Moffat, W. H., 1993. Long term residual effects of lead mining on man and grazing livestock within a rular community in Southern Scotland, PhD thesis University of Edingburgh, pp. 152-214.

Moza, P.N., Hustert, K., Feicht, E. and Kettrup, A., 1998. Photolysis of imidacloprid in aqueous solution. Chemosphere., 36(3): 497–502.

Muschal, M., Warne, M. S. J., 2003. Risk posed by pesticides to aquatic organisms in rivers of northern inland New South Wales, Australia. Human Ecological Risk Assessment, 9: 1765-1787.

Muir, D.C.G. and Baker, B.E., 1973. Pesticide residues in soil and foodstuff I. Chlorinated pesticides in cattle feed and milk produced in orchard and non-orchard areas. Pesticide Science, 4 (1): 113–119.

Muir, D.C.G., Rawn, G.P., Townsend, B.E., Lockhart, W.L. and Greenhalgh, R., 1985. Bioconcentration of cypermethrin, deltamethrin, fenvalerate and permethrin by Chironomus tentans larvae in sediment and water. Environmental Toxicology and Chemistry., 4: 51-61.

Mukherjee, I. and Gopal, V., 1993. Organochlorine pesticide residues in dairy milk in and around Delhi. AOAC International, 76 (2): 283-286.

Murphy, J.J., Headon, D.R. and Downey, W.K., 1977. Seasonal variations of copper and iron in Irish milk and butter. Dairy Research, 44: 325-334.

Muzzaralli, R.A., Eugeni, C.E., Tanfani, F., Garavia,G. and Pezzol, D., 1988. Trace element analytical chemistry in medicine and biology. Walter de Gruyter, Berlin.

National Research Council, 1975. Effects of genetic variance on nutritional requirements of Animals. Washington D. C. National Academic Press.

National Research Council, 1981. Effect of environment on nutrient requirements of domestic animals. Washington D. C. National Academic Press.

National Research Council,1990. Forestry research: A Mandate for Change, National Research Council. National Academy Press, Washington, D. C.

National Research Council, 1995. Nutrient requirement of laboratory animals. Fourth revised edition. Washington, D. C. National Academic Press.

National Research Council, 2001. Nutrient requirement of dairy cattle, 7th Edition, Washington, D. C. National Academy Press.

National Research Council, 2005. Committee on Minerals and Toxic Substances in Diets and Water for Animals, National Research Council (US) Subcommittee on Mineral toxicity in animals, 2005. Mineral tolerance of animals, Second edition, National Academic Press,Washington, D.C. 2005. 496 p.

Nasreen, S. Z., 2006. Monitoring of surface water ground water air and soil in Peshawar Basin against time the 3rd dimension. PhD thesis submitted to National Centre of Excellence in Geology, University of Peshawar.

Naz, A., Rehman, S.S. and Khan, S., 2011. Food chain contamination with heavy metals through wastewater irrigation. Environmental Sciences, 34(1):19-23.

Neathery, M. W., and Miller, W. J., 1975. Metabolism and toxicity of cadmium, mercury and lead in animals: A review. Journal of Dairy Science, 158(12): 1767-1781.

Nielsen, F. H., 1987. Nickel, Pp 245-274 in Trace elements n human and animals nutrition, vol 1, W. Mertz Ed, New York Academic Press.

NRC, 1990. Forestry research: A Mandate for Change, National Research Council. National Academy Press, Washington, D. C.

O'Brien E., 2010. Lead Advisory service News Volume 1 No. The Journal of The Lead Advisory Service ISSN 1440-0561.

O'Dell, G. D., Miller, W. J., Moore, S. L., King, W. A., Eller, J. C. and Jurecek, H., 1971. Effect of dietary nickel level on excretion and nickel contents of tissues in male calves. Animal Science, 32: 769-773.

Okada, S., Taniyama, M. and Ohba, H., 1982. Mode of enhancement in ribonucleic acid synthesis directed by Chromium-III bound deoxyribonucleic acid. Inorganic Biochemistry, 17: 41-49.

Onianwa, P.C., Adetola, I.G., Iwegbue, C.M.A., Ojo, M.F. and Tella, O.O., 1999. Trace heavy metals composition of some Nigerian beverages and food drinks. Food Chemistry, 66: 275-279.

Oscar, T. P. and Spears, J. W., 1988. Nickel-induced alteration of in vitro and in vivo ruminal fermentation. Animal Science, 66: 2313-2324.

Ostergaard, K., 1977. Cadmium in cigarettes. Acta Med. Scand., 202: 193-7.

Palmer, WE, Bromley, PT, and Brandenburg, RL. Wildlife & pesticides - Peanuts. North Carolina Cooperative Extension Service. Retrieved on 2007-10-11.

Park, J.E. and Park, K.,1992. Preventive and Social Medicine. Chap. Environment and Health. 12th Ed. Banarasidas Bhanot Publishers, Pg. 357-420.

Parkpian, P., Shing, T. L., Laortanakul, P. and Thunthaisong, N., 2003. Regional monintoring of lead and cadmium contamination in a tropical grazing land site, Thiland. Environmental Monitoring Assessment, 85: 157-173.

Pagliuca,G., Serraino,A., Gazzotti,T., Zironi,E., Borsari, A. and Rosmini, R., 2006. Organo phosphorus pesticide residues in Italian raw milk. Dairy Research, 73(3): 340-344.

Palikhe, B.R., 2002. 'Challenges and options of pesticides use: In context of Nepal' Landschaftsökologie und Umweltforschung, 38: 130-141.

Pennington, J.A.T., Wilson,D.B., Young,B.E., Johnson,R.D. and Vanderveen, J.E.,1987. Mineral content of market samples of fluid whole milk. American Dietary Association, 90: 1571- 1581.

Pennington, J.A.T. and Jones, J. W., 1987. Molybdenum, nickel, cobalt, vanadium and strontiumin total diets. American Diet Association 87: 1644-1650.

Perrin, D. J., Schiefer, B., Blakley, B. R., 1990. Chronic copper toxicity in a dairy herd. Canadian Veterinary Journal, 31: 629-632.

Philpott T., 2008. Conventional milk contains toxics, says the USDA

Phipps, A.D., 1976. Metals and metabolism. Clarendon press, Oxford.

Porru, S., and Alessio, L., 1996. The use of chelating agents in occupational lead poisoning. Occupational Medicine (London), 46(1): 41-48.

Pouls, G., 2009. The Chelation Controversy: How to Safely Detoxify Your Body and Improve Your Health And Well Being (Easy read Large Edition).

Profenophos (Ref:OMS 2004) Pesticide Properties Database PPDP. Environmental Fate EPA Profenofos Facts. United States Environmental Protection Agency. Prevention, Pesticides and Toxic Substances(7508C) EPA 738-F-00-005 July 2000.

Public Health Statement., 2011. Copper, Agency for toxic substances and disease registry toxicological profile.

Puls, R., 1994. Minerals levels in animal health 2nd Edition diagnostic data. Sherpa International Clear Book Canada.

Qin, L.Q., Wang, X.P., Li, W., Tong, X. and Tong, W.J., 2009. The Minerals and Heavy Metals in Cow's Milk from China and Japan. Health Sciences, 55 (2): 300-305. Quantitative Instrumental Analysis, 1998.

QuEChErs Information Booklet., 2010. United Chemists, UCT LLT. 2731/ Bartram Road Bristol. A 19007/USA

Quest, J.A., 1990. US Environmental Protection Agency, Washington, D.C., USA / 814.

Qureishi, M.S. and Khan, R., 2011. Evaluation of mineral contents and heavy metals of drinking water and their correlation with mineral contents in milk. Heavy metals in drinking water of dairy buffaloes at Peshawar, 2011-60.

Racke, KD., Laskowski, D. A. and Schultz, M.R., 1990. Persistence of chloropyriphos to enhanced biodegadation in soil. Agricultural and Food Chemistry; 38(6): 1430-1436.
Rafiq, M. and Sultani, M. I., 2004. Review of agro-livestock production in Pakistan with reference to WTO. Pakistan Journal of Science and Technology, 23:24-30.
Rahimi, E., and Rokni, N., 2008. Measurement of Cd residue in muscle, liver, and kidney slaughtered in Isfahan abattorior using graphite furnace atomic absorption spectrophotometry (GFAAS): A preliminary study. Iranian Journal of Veterinary Research, 9:174-177.
Raj, B. G., Patnaik, M.C., Babu, S.P., Kalakumar, B., Singh, M.V and Shylaja, J., 2006. Heavy metal contaminants in water-soil-plant-animal contionuum due to pollution of Musi river around Hyderabad in India. Indian Journal of Animal Sciences, 76: 131-133.
Rajaganapathy, V., 2009. Biomonitoring pollution in livestock production systems in industrialized areas of Palakkand Kerela India. Proceedings of National Seminar of Wealth from livestock and Agricultural Waste, Nov 12-13, Veterinary College and Research Institute, Namakal Tamil Nadu, India, p 80-82.
Reilly, C., 1969. The uptake and accumulation of copper by Becium homblei. New Phytol., 68, 1081-1087.
Reilly, C., Sudarmadji, S. and Sugiharto, E., 1989. Mercury and arsenic in soil, water and foods of the Deing Plateau, Java. Proceeding Nutrition Society Australia Contamination 5: 161-232
Reilly, C., 1991. Metal Contamination Of Food. Chap. The Metals We Consume. Pg 1-20. 2nd Edition. Elsevier Science Publishers Limited.
Reisbeck, M.F., Roger, S.S. and Michael, A.S., 2006. Modest copper supplementation blocks molybdenosis in cattle. Journal of Venality Diagnostic Investigation, 18: 566-572.
Renata, P., Lopes, Ana, P.F.M., de Urzedo, Celsia, C. Nascentes and Augusti, R., 2008. Degradation of insecticides thiamethoxam and imidacloprid by zero valent metals exposed to ultrasonic irradiation in water medium. Electro spray ionization mass spectrometry monitoring. Rapid Communications in Mass Spectrometry, 22:3472-3480.
Richard, B. P., 2001. Ecosystems and human health: toxicology and environmental hazards 2nd edition. Lewis Publications, Medical, 328p.
Rietz, D., 2010. Dangers of milk and dairy products- The Facts.
Roberts, T. R. and Hutson, D. H., 1999. Imidacloprid. Metabolic Pathways of Agrochemicals – Part 2: Insecticides and Fungicides; The Royal Society of Chemistry: Cambridge, UK: pp 111-120.
Rockets, R., 2007. Down On The Farm Yields, Nutrients And Soil Quality. Scienceagogo.com.
Rolfes, S.R., Pinna, K. and Whitney, E., 2008. Understanding normal and clinical nutrition. Cengage Learning, Medical. 925p.
Rothwell, J. T., Burnett, T.J., Hacket, K., Chevis, R and Lowe, L.B., 2001. Residues of zeta-cypermethrin in bovine tissues and milk following pour-on and spray application. Pest Managament Science, 57(11): 993-999.
Roussel, A.M., Richard, A.A. and Favier, A., 2000. Trace elements in man and animals 10. Springer. 1172 p.
Rudd, R. L. and Genelly, R. E., 1956. Pesticides; their use and toxicity in relation to wildlife. Calif. Dep. Fish and Game Bulletin, 7: 207 pp.
Russell, L.H., 1978. Toxicity of heavy metals in the environment. Heavy metals in foods of animal origin. OEHME, F.W, M.Dekker New York. p. 3-23.

Saad, M. A. H. and Fahmy, M. A., 1996. Heavy metal pollution in coastal Red Sea Waters, Jeddah. Journal of King Abdul Aziz University Marine Science, 7: 67-74.
Sadler, P.J., 1982. Trace elements in human and animal nutrition. by E. J. Underwood. Academic Press, New York. 4th Edition . p 545.
Sakata, S., Nobuyoshi, M., Matsuda, T. and Miyamoto, J., 1986. Degradation and leaching behavior of pyrethroid insecticide cypermethrinin soils. Pesticide Science 11: 71-79.
Salas, J.H, González, M.M., Noa, M., Pérez, N.A., Díaz, G., Gutiérrez, R., Zazueta, H and Osuna, I. 2003. Organophosphorus pesticide residues in Mexican commercial pasteurized milk. Agriculture and Food Chemistry, 51(15): 4468-71
Sarkar, M. A., Roy, S.M. and Chowdhury, A., 2001. Persistence and metabolism of imidacloprid in different soils of west Bengal. Pest Management Science 57: 598-602.
Sarwar, M., Khan, M. A., Mahr-un-Nisa, and Iqbal, Z., 2002. Dairy industry in Pakistan; A Scenario. International Journal of Agriculture and Biology 3: 420-428.
Sassine, A., Maura, S., Leo, V.M. and Buustillos, O.V., 2004. Cypermethrin residues determination in the milk of a lactating dairy cow by gas chromatography ion trap mass spectrometry. Analysis of Toxicology 28 (4): 238-241.
Schenck,F.J. and Wong, J.W., 2008.Determination of Pesticides in Food of Vegetal Origin. Chapter 6. Analysis of pesticides in food and environmental samples.Edited by José L. Tadeo. CRC Press.
Schroeder, M. E. and Flattum, R. F., 1984. The Mode of Action and Neurotoxic Properties of the Nitroethylene Heterocycle Insecticides. Pesticides Biochemistry Physiology 22: 148-160.
Schock, M.R. and Neff, C.H., 1988. Trace metal contamination from brass fittings. American Water Works Association 80: 47-56
Schwarz, K. and Mertz, W., 1959. A glucose tolerance factor and its differentiation from factor 3. Archives Biochemistry and Biophysics, 85: 292-295.
Selinus, O., 2005. Essentials of medical geology: impacts of the natural environment on public health.
Sharma, N., Kowale, B. N. and Joshi, H. B., 1982. Effect of cooking and storage on neutral lipids in broiler and quail. Food Science and Technology, 19; 7-10.
Sharma, R. P., Street, J. C., Verma, M. P. and Shupe, J. L., 1979. Cadmium uptake from feed and its distribution to food products of livestock. Environmental Health Prespectives, 28: 59-66.
Sheets, L. P., 2002. The Neonicotinoid Insecticides. Handbook of Neurotoxicology; Massaro, E. J., Ed.: Humana Press: Totowa, NJ, Vol. 1, Chapter 6, pp 79-87.
Siddiqui, S., 2011. Spatial distribution of trace and major elements in the hilly soils of Lesser Himalayan Muzaffarabad-Pakistan. Soil Science, 176 (7): 372-383.
Siddiqui, S., and Khattak, R. A., 2010. Trace elements fractionation in the calcareous soils of Peshawar-Pakistan. Soil and Environment, 29: 148-158.
Sidhu, P.K., Sandhu, B. S., Singh, J. and Kwatra, M. S., 1994. Lead toxicosis in bovine due to industrial pollution in Punjab. Indian Journal of Animal Science, 64: 1359-1360.
Siegfried, B.D., 1993. Comparative toxicity of pyrethroid insecticides to terrestrial and aquatic insects. Environmental toxicology and chemistry, 12: 1683-1689.
Simsek, O., Gultekin, R., Oksuz, O. and Kurultay, S., 2000. The effect of environmental pollution on the heavy metal content of raw milk. Food Nahrung, 4 (5): 360 -363.
Smart, G. A. and Sherlock, J. C., 1987. Nickel in foods and diet. Food Addi. Contam., 4: 61-71.

Smith, G.N., 1966. Basic studies of Dursban insecticide. Down to Earth, 22(2): 3-7.
Smith, G.N., 1968. Ultraviolet light decomposition studies with Dursban and 3,5,6- trichloro-2-pyridinol. Economic Entomology 61(3):793-799.
Smith, G.N., Watson, B.S. and Fischer, F.S.,1966. The metabolism of (^{14}C) O,O-diethyl O-(3,5,6-trichloro-2-pyridyl)phosphorothioate (Dursban) in fish. Econmic Entomology 59(6):1464-1475.
Smith, G.N., Watson, B.S. and Fischer, F.S., 1967a. Investigations on Dursban insecticide. Uptake and translocation of (^{36}Cl) O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate by beans and corn. Agriculture Food Chemistry, 15(1): 127-131.
Smith, R. M., Leach, R. M., Muller, L. D., Griel, L. C. and Baker, D. E., 1991. Effects of long term dietary cadmium chloride on tissue, milk and urine mineral concentrations of lactating dairy cows. Animal Sciences, 69: 4088-4096.
Solis, C., Isaac, K., Mireles, A and Hernandez, M., 2009. Determination of trace metals in cow's milk from waste water irrigated areas in Central Mexico by chemical treatment coupled to PIXE. Microchemical Journal, 91 (1): 9-12.
Somasundaram, J., Krishnasamy, R. and Savithri, P., 2005. Bio transfer of heavy metals in Jersey cows. Indian Journal of Animal Science, 75: 1257-1260.
Spears, J., 1984. Nickel as a new trace element in the nutrition of domestic animals. Animal Sciences, 59: 823-835.
Srivastava, S., Narvi, S.S. and Prasad, S.C., 2008. Organochlorines and Organophosphates in Bovine milk samples in Allahabad region. International Journal of Environment, 2(2): 165-168.
Stangle, G. I., Schwarz, F. J., Muller, H., and Kirchgessner, M., 2000. Evaluation of the cobalt requirement of beef cattle based on vitamin B12 folate, homocysteine and methylmalonic acid. British Journal of nutrition, 84: 645-653.
Steiniger,D., Lu,G., Butler,J., Phillips, E. and Fintschenko., 2010. Determination of Mutiresidue pesticides in Green Tea by using a modified QuEChERs Extraction and ion Trap Gas Chromatography/ Mass Spectrometry. Journal of AOAC International, 93(4):1169-1179.
Stettler,LE., Procter, JE., Platek SF., Carolan, RJ., Smith, RJ. And Donaldson, HM., 1988. Fibrogenicity and carcinogenic potential of smelter slags used as abrasive blasting substitutes; Toxicology and Environmental Health, 25 (10): 35 – 56.
Stellman, J. M., 1998. Encyclopaedia of occupational health and safety.4th ed, International labor office, Geneva Switzerland.
Stokinger, H. E., 1963. Nickel in Industrial Hygiene and Toxicology, Vol. II (Interscience, New York).
Swarup, R.C., Patra, R., Naresh, P., Kumar, P. and Shekhar, P., 2005. Blood lead levels in lactating cows reared around polluted localities, transfer of lead into milk. Science of the Total Environment, 347: 106-110.
Sugitate, K. and Kana, M., 2008. Multi-residue Analysis of Pesticides in Food using GC/MS/MS with the TSQ Quantum GC. Thermo Fisher Scientific, Yokohama, Japan.
Sunderman, F. W., 1965. Nickel Toxicity. American Journal of Clinical Pathology 44: 182- 200.
Suttle, N. F., and Field. A. C., 1970. Effect of intake of copper, molybedenum and sulphate on copper metabolism in sheep. IV Production of congenital and delayed swayback. Composition Pathology, 79(4):453-464.
Suttle, N. F., 2010. Mineral Nutrition of Livestock, 4th Edition. CABI, 587p.

Tajkarimi, M., Faghih, M. A., Poursoltani, H., Nejad, A. S., Motallebi, A. A., Mahdavi, H., 2008. Lead residue levels in raw milk from different regions of Iran. Food Control, 19: 495-498.

Takahashi, N., Mikami, N., Matsuda, T. and Miyamoto, J., 1985. Photodegradation of the pyrethroid insecticide cypermethrin in water and on soil surface. Pesticide Science, 10: 629-642.

Tariq, M. I., Afzal, S., Hussain, I. and Sultana, N., 2007. Pesticides exposure in Pakistan; A review. Environment International, 33 (8): 1107-1122.

Thyssen, J. and Machemer, L., 1999. Imidacloprid: Toxicology and Metabolism. Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor; Yamamoto, I.; Casida, J. E., Eds.;Springer-Verlag: Chapter 9, p 213-222.

Tiffany, M. E., and Spares, J. W., 2005. Differential responses to dietary cobalt in finishing steers fed corn vs. barley-base diets. Animal Science, 83: 2580–2589.

Toepfer, E.W., Mertz,W., Polansky,M.M., Roginski, E.E. and Wolf, W. R., 1977. Preparation of chromium containing material of glucose tolerance factor activity from brewer's yeast extracts and by synthesis. Agriculture Food Chemistry, 25: 162-166.

Tomizawa, M. and Casida, J. E., 1999. Minor structural changes in nicotinoid insecticides confer differential subtype selectivity for mammalian nicotinic acetylcholine receptors. Britain Journal of Pharmacol, 127: 115–122.

Tomlin, C. D. S., 2006. The Pesticide Manual, A World Compendium, 14th ed.; British Crop Protection Council: Surry, England, pp 598-599.

Toskes, P., Smith, G. W. and Conrads, M.E., 1973. Cobalt absorption in sex linked anaemic mice. American Journal of Clinical Nutrition, 26: 435-437.

Tripathi, R.M., Raghunath, R., Sastry, V.N. and Krishnamoorthy, T.M., 1999. Daily intake of heavy metals by infants through milk and milk products. The Science of Total Environment, 227: 229 – 235.

Underwood, E. J., 1977. Trace elements in human and animal nutrition, fourth edition, Academic Press, London.

Underwood, E. J., and Harvey, R. J., 1937. Enzootic marasmus: The cobalt content of soils, pastures and animal organs. Australia Veterinary Journal, 13: 183-189.

Underwood, E. J., 1991. The mineral nutrition of livestock. Common Wealth Agriculture Bureaux, London.

Underwood, E. J. and Suttle, F., 1999. The mineral nutrition of livestock, Third Edition, CAB International Wallingford, UK.

USDA Agricultural Research Service., 1995. ARS Pesticide Properties.

U.S. Environmental Protection Agency, 1985. Updated mutagenicity and carcinogenicity assessment of cadmium: addendum to the health assessment document for cadmium. Report No. EPA/600/8-83/025F, Office of Health and Environmental Assessment, Office of Research and Development, Washington, DC.

USEPA Manual., 2009. Nutrient control manual.

Varo, P. and Koivistoinen, P. 1980. Mineral composition of Finnish foods XII. General discussion and nutritional evaluation. Acta Agriculture Scandinavica Supplementum, 22:165-70.

Varo, P., Nuurtarno, M., Saari, E. and Koivistoinen, P., 1980. Mineral elements composition of Finnish foods. VII Dairy Products, eggs, margarine. Acta Agriculture Scord Supply, 22:115-126.

Vijverberg, H.P.M. and Van den Bercken, J., 1990. Neurotoxicological effects and the mode of action of pyrethroid insecticides. Critical Reviews in Toxicology, 21(2): 105-126.

Vos,G., Hovens, J. P. C. and Delft, W. V., 1987. Arsenic, lead, cadmium and mercury in meat liver and kidneys of cattle slaughtered in the Netherlands during 1980 – 85. Food Additive Contamination, 4: 73 – 88.

Vreman, K., Vander veen, N. G., Vander Molen, E. J. and De Rug, W. J., 1986. Transfer of cadmium, lead, mercury and arsenic from feed into milk and various tissues of dairy cows: chemical and pathological data. Netherland Journal of Agriculture, Science 34: 129-144.

Waliszewski, S.M., Pardio, V.T., Waliszewski, K.N., Chantiri, J.N., Aguirre, A.A., Infanzon, R.M., and Rivera, J., 1997. Organochlorine pesticide residues in cow's milk and butter in Mexico. Science of the Total Environment, 208 (1-2): 127-132.

Walker, M.H. and Keith, L.H., 1992. EPA's Pesticide Fact Sheet Database. Lewis Publishers, Chelsea, MI.

Walley F, Taylor A. and Lupwayi, 2006. Herbicide effects on pulse crop nodulation and nitrogen fixation. Farm Tech 2006 Proceedings 121-123.

Wang, T., 2005. Encyclopaedia Of Chromatography, 2nd edition. CRC Press. Pages 1401-1402. Print ISBN:978-0-8247-2785-7.

Wapnir, R. A., Devas, G. and Solars, C. V., 1993. Inhibition of intestinal copper absorption by divalent cations and low molecular weight ligands in the rat. Biological Trace Element Research, 36;291-305.

Ware, G. W. and Whitacre, D. M., 2004. The Pesticide Book; MeisterPro Information Resources: Willoughby, OH. pp 70-71.

Webster, R., and Rivoirard, J., 1991. Copper and cobalt deficiency in soil: a study using disjunctive kriging. In: Journal of Geostatistics, Proceedings of the Days of Geostatistic, 1: 205–233.

Whitman, W.E., 1978. Interaction between structural materials in food plant and foodstuffs and cleaning agents. Food Progress, 2: 1-2.

Whitney, R. and Lyle, C.R.,2007. Rapid multiresidue Pesticides Analysis Using Fast-GC/MS and Chromatogram Deconvolution Software. Shimadzu scientific Instruments Columbia.

Williams, C. H. and David, D. J., 1973. Heavy metals in Australian agricultural soils. Australia Journal of Soil Research, 11: 43-50.

Winter, W. J., Siebert, D. B., and Kuchel, R. E., 1977. Cobalt and copper therapy of cattle grazing improved pastures in northern Cape York Peninsula. Australian Journal of Experimental Agriculture and Animal Husbandry, 17; 10-15.

Wismer, T., 2004. Novel Insecticides. Clinical Veterinary Toxicology; Plumlee, K. H., Ed.; Mosby: St. Louis, MO: pp. 184-185.

WHO 1990. The WHO recommended classification of pesticides by hazard and guidelines to classification 1990-1991 (WHO/PCS/90.1).

World Health Organization, 1976. Environmental Lead Criteria 3: Lead (WHO Geneva).

WHO., 1999. Maximum residue limits of pesticides.

Wong, J.W., 2007. Multiresidue Analysis of Pesticides in Fresh Produce using QuEChERS concepts, GC, and GC-MS/SIM. Food and Drug Administration Center for Food Safety and Applied Nutrition College Park, MD, Florida. Pesticide Residue Workshop.

Wong, S.K. and Lee, W.O., 1997. Survey of organochlorine pesticide residues in milk in Hong Kong 1993-1995. AOAC International, 80 (6): 1332-1335.

Wright, C.G., R.B. Leidy and H.E. Jr. Dupree 1991). Chlorpyrifos in the air and soil of houses four years after its application for termite control. Bulletin Environmental Contamination Toxicology 46(5): 686–689.

Yamagata, N., Muratan, S. and Morii, T., 1962. The cobalt content of the human body. Radiation Research, 3: 4-8.

Yang, W.Z., Mowar, D.N., Subiyatno,A and Liptrap, R.M., 1996. Effects of chromium supplementation on early lactation performance of Holstein cows. Canadian Journal of Animal Science, 76: 221-230.

Zahoorullah, Akhtar, T. and Zai,S., 2003. Quality of drinking water in rural Peshawar. Pakistan Journal of Medical Research, 42 (3): 85-89.

ANNEXURES

ANNEXURE 1

TABLE 1.1. CONCENTRATION (mg L^{-1}) OF HEAVY METALS IN RAW MILK SAMPLES

Area 1 rural	Cd	Cr	Co	Cu	Ni	Pb
1	0.15	0.001	0.007	2.15	3.2	7.8
2	0.0006	0.001	0.007	2.45	1.65	0.012
3	0.0006	0.001	0.007	0.78	0.004	6.4
4	0.45	0.034	1.75	0.69	0.85	0.012
5	0.63	0.024	1.5	1.1	0.65	0.012
6	0.25	0.009	0.72	0.38	0.004	0.012
7	0.42	0.009	0.48	1.8	1.9	3.8
8	0.91	0.001	2.42	0.65	0.004	0.012
9	0.30	0.001	0.96	0.54	0.004	0.012
10	0.42	0.001	0.007	1.73	0.004	4.28
Area 2 urban	**Cd**	**Cr**	**Co**	**Cu**	**Ni**	**Pb**
11	0.65	0.06	1.15	3.2	1.28	0.012
12	0.95	0.009	0.007	2.9	1.1	0.012
13	1.05	0.007	0.007	1.6	0.004	8.40
14	0.84	0.006	0.96	1.2	0.2	0.012
15	0.3	0.001	2.6	0.85	0.004	7.28
16	0.98	0.001	0.007	0.74	1.4	0.012
17	0.36	0.001	0.007	0.82	0.004	0.012
18	0.49	0.003	0.007	1.35	0.05	0.012
19	0.66	0.001	0.007	2.14	0.004	6.42
20	0.74	0.001	0.86	1.9	0.004	0.012
Area 3 urban	**Cd**	**Cr**	**Co**	**Cu**	**Ni**	**Pb**
21	0.25	0.008	0.45	1.36	0.65	0.012
22	0.29	0.006	0.38	1.6	1.82	11.36

23	1.15	0.001	0.007	0.89	0.004	0.012
24	0.9	0.002	0.007	0.94	0.004	0.012
25	1.05	0.061	0.29	2.30	0.12	2.48
26	0.75	0.074	0.007	1.15	0.004	0.012
27	0.28	0.001	0.18	1.68	1.34	0.012
28	0.37	0.14	0.007	0.75	0.004	9.67
29	0.46	0.10	0.007	0.88	0.004	0.012
30	0.52	0.12	0.007	0.14	0.67	0.012
Area 4 urban	**Cd**	**Cr**	**Co**	**Cu**	**Ni**	**Pb**
31	0.85	0.001	0.007	0.98	0.95	5.8
32	0.72	0.04	0.48	1.67	0.64	0.012
33	0.34	0.8	0.007	1.23	0.004	0.012
34	0.48	0.001	0.1	0.87	0.004	4.76
35	0.29	0.009	1.34	0.71	1.78	0.012
36	0.12	0.008	0.007	1.73	0.004	0.012
37	0.22	0.04	0.007	2.32	0.004	3.4
38	0.7	0.001	0.96	1.40	2.39	0.012
39	0.63	0.001	0.007	0.68	0.004	0.012
40	0.31	0.001	0.007	0.50	0.55	5.6
Area 5 rural	**Cd**	**Cr**	**Co**	**Cu**	**Ni**	**Pb**
41	0.26	0.04	1.26	0.24	0.34	0.012
42	0.10	0.03	0.28	0.86	0.42	8.7
43	0.31	0.001	0.007	1.29	0.004	0.012
44	0.93	0.001	0.007	1.38	1.29	0.012
45	0.84	0.001	0.63	0.66	0.004	6.9
46	0.38	0.102	0.007	1.67	0.96	0.012
47	0.65	0.140	0.78	2.12	0.004	0.012
48	0.57	0.001	0.007	0.71	0.004	4.2
49	0.12	0.001	0.007	0.38	1.01	3.87
50	0.19	0.001	0.007	0.64	0.004	0.012

TABLE 1.2. CONCENTRATION (mg L^{-1}) OF HEAVY METALS IN PASTEURIZED MILK

No. of samples	Cd	Cr	Co	Cv	Ni	Pb
51	0.45	4.8	0.007	2.8	0.65	1.8
52	1.1	0.001	2.10	1.6	0.82	0.012
53	0.38	1.4	1.8	1.32	0.004	0.012
54	1.46	0.001	0.45	0.72	1.45	0.012
55	0.35	0.001	0.96	0.34	0.004	4.34
56	0.98	4.6	1.1	0.93	0.73	0.012
57	1.59	3.7	0.007	1.53	0.004	0.012
58	0.74	0.001	0.87	0.86	0.004	2.36
59	0.86	2.68	0.007	0.74	1.46	0.012
60	0.96	2.5	1.32	1.1	0.78	0.012

TABLE 1.3. CONCENTRATION (mg L^{-1}) OF HEAVY METALS IN DRY MILK

No. of samples	Cd	Cr	Co	Cu	Ni	Pb
61	0.52	3.6	1.74	1.4	0.4	1.67
62	0.96	1.80	0.65	0.72	1.45	0.012
63	0.34	0.001	0.97	0.53	0.004	0.012
64	1.32	0.34	0.007	1.6	1.29	3.82
65	1.64	0.001	1.32	0.93	0.004	0.012
66	0.73	0.001	0.007	0.86	1.68	2.79
67	0.24	2.76	2.10	2.8	0.78	0.012
68	0.78	0.001	0.007	0.83	0.004	4.3
69	1.62	1.93	0.84	1.53	0.54	0.012
70	0.47	0.001	0.007	0.64	0.004	0.012

TABLE 1.4. CONCENTRATION (mg kg^{-1}) OF HEAVY METALS IN ANIMAL FEED

Farm No	Cotton Khal	Cd	Cr	Co	Cu	Ni	Pb
1	Urban	0.32	4.8	0.60	80.92	2.12	3.8
2	Urban	0.39	5.0	0.42	85.24	1.93	4.2
3	Urban	0.30	4.7	0.97	83.56	2.84	4.6
4	Rural	0.38	5.4	0.34	84.20	2.65	3.5
5	Rural	0.36	5.2	0.81	82.67	2.01	4.2
	Sugar Cane Khal						
1	Urban		19.79	0.02	4.82	1.12	2.91
2	Urban		20.83	0.08	3.68	1.19	2.87
3	Urban		22.52	0.06	5.92	1.17	3.12
4	Rural		21.84	0.01	6.31	1.04	3.48
5	Rural		21.96	0.01	6.27	2.01	2.84
	Wheat Bran						
1	Urban	0.10	1.92	0.06	0.22	0.21	4.28
2	Urban	0.04	1.42	0.01	0.37	0.62	5.67
3	Urban	0.15	1.80	0.08	0.42	0.91	5.23
4	Rural	0.08	2.12	0.02	0.58	1.73	6.94
5	Rural	0.09	2.01	0.01	0.65	1.36	6.48
	Wheat						
1	Urban	0.13	8.14	0.03	0.18	6.30	5.10
2	Urban	0.14	8.14	0.03	0.19	6.21	4.64
3	Urban	0.16	8.20	0.09	0.23	6.30	5.14
4	Rural	0.09	8.64	0.01	0.34	6.84	5.67

5	Rural	0.15	8.81	0.02	0.32	5.83	6.48
	Area 5	0.13	8.25	0.06	0.28	7.62	6.94
	Maize						
1	Urban	0.22	22.1	0.09	0.18	0.92	7.97
2	Urban	0.20	24.4	0.16	0.19	1.08	8.02
3	Urban	0.28	27.0	0.15	0.28	1.95	8.48
4	Rural	0.21	26.3	0.19	0.20	2.3	7.63
5	Rural	0.29	23.5	0.14	0.10	2.4	8.23
	Mixed						
1	Urban	0.25	4.3	0.21	0.28	0.86	4.12
2	Urban	0.30	3.9	0.34	0.39	1.90	4.48
3	Urban	0.29	3.2	0.42	0.40	0.92	3.87
4	Rural	0.33	4.1	0.65	0.31	nd	nd
5	Rural	Nd	Nd	Nd	nd	nd	nd

ANNEXURE 2

TABLE 2.1. CONCENTRATION (mg L^{-1}) OF PESTICIDES IN MILK

RAW MILK	Cypermecthin	Chloropyrphos	Profeno Phos.
1	0.1817	1.68×10^{-4}	9.16×10^{-3}
2	0.367	0.757	1.53×10^{-3}
3	0.366	0.724	0.0133
4	0.0017	0.059	1.72×10^{-3}
5	0.0026	1.20×10^{-3}	3.36×10^{-3}
6	0.289	0.399	3.8×10^{-3}
7	0.049	0.328	1.04×10^{-3}
8	0.8765	1.68×10^{-3}	1.02×10^{-3}
9	0.056	9.21×10^{-3}	0.032
10	0.066	6.81×10^{-3}	1.65×10^{-3}
11	0.223	0.034	1.65×10^{-3}
12	0.268	0.036	7.9×10^{-4}
13	0.288	7.62×10^{-3}	6.3×10^{-4}
14	0.235	0.029	7.12×10^{-4}
15	0.280	0.029	5.2×10^{-4}
16	0.254	0.020	4.5×10^{-4}
17	0.197	0.026	2.8×10^{-4}
18	0.126	0.042	3.4×10^{-4}
19	0.126	0.033	4.7×10^{-4}
20	0.088	0.037	2.09×10^{-4}
21	0.086	0.158	1.32×10^{-3}
22	0.086	0.018	1.09×10^{-3}

23	0.067	0.023	1.57×10^{-3}
24	0.077	0.018	1.9×10^{-5}
25	0.057	0.017	1.57×10^{-3}
26	0.043	0.016	1.66×10^{-3}
27	0.232	0.016	2.05×10^{-3}
28	0.260	0.033	1.70×10^{-3}
29	0.097	0.03	1.9×10^{-3}
30	0.105	0.0002	1.5×10^{-3}
31	0.372	3.07×10^{-3}	2.7×10^{-3}
32	0.399	0.046	2.7×10^{-3}
33	0.302	0.046	1.78×10^{-3}
34	0.344	0.041	2.12×10^{-3}
35	0.314	0.054	1.49×10^{-3}
36	0.294	0.040	1.92×10^{-3}
37	0.328	0.066	2.8×10^{-3}
38	0.373	0.43	2.6×10^{-3}
39	0.247	0.089	1.43×10^{-3}
40	0.242	4.15×10^{-3}	1.6×10^{-3}
41	0.340	0.038	1.85×10^{-3}
42	0.273	0.055	2.4×10^{-3}
43	0.280	0.045	3.02×10^{-3}
44	0.064	0.42	2.59×10^{-4}
45	0.225	0.269	1.8×10^{-3}
46	0.204	0.057	1.3×10^{-3}
47	0.053	0.063	1.59×10^{-3}
48	0.184	0.049	1.62×10^{-3}
49	0.165	0.032	1.58×10^{-3}
50	0.216	0.044	1.82×10^{-4}

PASTEURIZED MILK			
51	0.067	6.94×10^{-3}	3.2×10^{-4}
52	0.142	5.81×10^{-4}	1.01×10^{-4}
53	0.038	5.27×10^{-3}	1.49×10^{-4}
54	0.234	4.08×10^{-4}	6.7×10^{-4}
55	0.183	2.86×10^{-3}	1.26×10^{-3}
56	0.281	1.69×10^{-3}	1.48×10^{-3}
57	0.066	2.66×10^{-4}	2.69×10^{-3}
58	0.194	6.04×10^{-3}	5.42×10^{-4}
59	0.108	3.39×10^{-2}	3.05×10^{-4}
60	0.094	1.52×10^{-2}	2.32×10^{-3}
DRY MILK			
61	0.043	4.41×10^{-3}	2.7×10^{-4}
62	0.00429	3.29×10^{-3}	2.45×10^{-4}
63	0.094	6.7×10^{-3}	5.22×10^{-4}
64	0.031	2.4×10^{-3}	2.75×10^{-4}
65	0.082	1.9×10^{-2}	1.89×10^{-4}
66	0.0046	4.3×10^{-2}	8.7×10^{-5}
67	0.0029	6.7×10^{-3}	6.99×10^{-5}
68	0.042	5.24×10^{-3}	3.33×10^{-4}
69	0.026	4.89×10^{-3}	1.18×10^{-4}
70	0.067	4.10×10^{-3}	3.39×10^{-3}

TABLE 2.2. CONCENTRATION (mg kg^{-1})OF PESTICIDES IN ANIMAL FEED

	Cypermethrin	Chlorophyriphos	Profenophos
COTTON KHAL			
Sample 1	0.030	1.21×10^{-3}	3.29×10^{-4}
Sample 2	0.020	1.46×10^{-3}	2.61×10^{-3}
Sample 3	0.039	4.21×10^{-3}	2.5×10^{-4}
Sample 4	0.015	3.69×10^{-3}	3.5×10^{-4}
Sample 5	0.028	2.92×10^{-3}	2.4×10^{-4}
SUGAR CANE KHAL			
Sample 1	0.026	3.59×10^{-3}	2.48×10^{-4}
Sample 2	0.014	6.21×10^{-3}	2.61×10^{-3}
Sample 3	0.038	0.0116	2.56×10^{-4}
Sample 4	0.032	4.24×10^{-3}	3.51×10^{-4}
Sample 5	0.016	3.5×10^{-3}	5.31×10^{-4}
MIXED DIET			
Sample 1	0.048	2.59×10^{-3}	5.6×10^{-4}
Sample 2	0.041	4.86×10^{-3}	4.8×10^{-4}
Sample 3	0.036	3.54×10^{-3}	4.2×10^{-4}
Sample 4	0.023	3.48×10^{-3}	3.7×10^{-4}
Sample 5	0.022	2.48×10^{-3}	3.1×10^{-4}