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**Contaminant Assessment of Cattle Feed and Milk and Associated Health Impacts on Consumers in H-11/4 Slums, Islamabad**

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**Abstract**

Milk is a crucial part of a balanced diet, providing essential minerals, vitamins, and protein. However, contamination by heavy metals and microorganisms poses serious health risks, especially to children. In slum areas, livestock farming provides food and income, with milk being a valuable product. But, the dairy value chain lacks information on proper handling, quality control, and marketing, leading to contamination. The present study conducted at the H-11/4 slums of Islamabad, assessed the health risks associated with specific biological and chemical contaminants in dairy cattle feed and milk. Utilizing total plate count (TPC), coliform plate count (CPC), and *Salmonella* and *Shigella* plate count (SSPC), eight milk and eight feed samples from four dairy farms were examined for microbial contamination. All sixteen samples revealed significant levels of coliforms, total bacteria, and *Salmonella* and *shigella* species. The mean CPC was found to be 6.58 (log<sub>10</sub> cfu/ml), mean TPC was 6.25 log<sub>10</sub> cfu/ml, and a mean SS count of 5.04 log<sub>10</sub> cfu/ml. The mean nitrate levels in milk and feed (dry and wet) were 11.83 mg/L, 11.67 mg/L, and 12.22 mg/L. Similar to this, the mean sulphate levels in milk and feed (dry and wet) were 24.3 mg/L, 27.02 mg/L, and 15.39 mg/L. Additionally, ICP-OES was used to assess fifteen (15) major and minor elements. The risk assessment of milk contaminants indicated HQ and CR of Ba, Cd, Cu, Ni, As, and Pb, surpassing safety limits and presenting considerable health risks including potential carcinogenic risks. Study also found a significant link between feed and milk elemental contents, suggesting that feed contamination is a primary source of contaminants. An association between bacterial and nutritional levels indicated that contamination may result from poor milk processing and unhygienic farm conditions. The results of surveys revealed inadequate hygiene practices in dairy management, characterized by the use of tube well water for sanitation and the storage of milk at ambient temperature, fostering bacterial proliferation. Farmers were uninformed about appropriate animal husbandry, resulting in numerous cattle diseases and dependence on expensive private doctors for treatment. Overall, the milk exhibited signs of poor hygiene and indicates a possible source of milk-borne diseases. All the cattle dairy farms must ensure preventive measure as well good hygiene practice during the milk production, handling, storage, and delivery process to avoid any contamination.

**Practical Application**

The findings of this research shed light on the potential risks associated with the quality of milk produced in slum areas. This information can be used by policymakers, dairy farmers, and relevant authorities to develop and implement strategies that improve milk hygiene, production practices, and quality control measures. By addressing the issues of microbial contamination and the

presence of heavy metals, efforts can be made to ensure safer milk for consumers, especially vulnerable populations like children, and mitigate the risk of milk-borne illnesses in slum communities.

## **Introduction**

Milk and its derivatives include one of the essential constituents of the human diet, mainly because of the mineral, vitamins, and protein contributions in a balanced proportion (1). Milk consists of a diverse range of nutrients like proteins, fats, and carbohydrates which are all the nutrients required for growth and the maintenance of bodily health, they are complete food for people from infancy to old age (2). Milk from cows, buffaloes, goats, sheep, and camels has been consumed by humans for thousands of years (3). Therefore, the contamination of milk by heavy metals and microorganisms can pose a serious public health problem due to the different health conditions and diseases among consumers, especially in children (4). The quality of milk and dairy products is influenced by various factors, including environmental conditions, production methods, storage practices, chemical composition, fat and protein levels, and, crucially, the management of the feed provided to the animals (5). Milk consumption is responsible for 4% of all foodborne zoonotic diseases globally, according to the World Health Organization's (WHO) Global Burden of Foodborne Disease initiative (6). It is noteworthy that the low- and middle-income countries bear the most burden associated with these diseases (7). Pathogenic bacteria present in milk are responsible for up to 90% of all illnesses related to dairy products (8). Due to its high nutritional value and the production and processing methods used in milk production, a variety of pathogenic bacteria, that could infect people, are able to contaminate it (9). As a result, milk is recognized as a major effective means of spreading disease-causing agents to people (10). Dairy products have been characterized as a major contributor to morbidity and mortality globally. According to estimates, dairy products contributed 20 DALYs (Disability Adjusted Life Years) per 100,000 persons in 2010 (11). This accounts for about 12% of the burden of food-borne diseases associated with animal source food (12). Toxic metal contamination in milk poses a serious risk to human health, especially for newborns and young children who consume a substantial amount of milk (13). Heavy metal poisoning of the environment has led to the feed origin of hazardous substances seen in animal milk and meat (14). Heavy metals are bio-transferred, bioaccumulated, and biomagnified in the bodies of the animals in food chains and food webs and either build up or harm them. Raw milk from various parts of the world contains 38 micro and trace components (15-16). These mineral concentrations in raw cattle milk can change based on a number of variables, including the cows' milking cycle, their overall general health, seasonal fluctuations, climatic circumstances, the composition of their annual feed, and environmental contamination (17-18). Because heavy metals are not biodegradable, they tend to accumulate and intensify their effects at various points along the production chain (19). Many people live in slums, which are unmanaged squatter settlements. These congested areas lack basic life and health services (20). Animal and human health are put at risk by livestock kept in these surroundings. Poverty, food security, livestock keeping, and the environment are all interlinked in slums (21). Slums often practice small- to medium-scale, low-tech cattle farming. While a few animals in slums are kept in inappropriate restricted areas shared with people, others are free to roam and graze. Depending on the type and scope, this practice might range from simple to small- to medium-sized businesses with hundreds of small animals (22). Slum livestock farming is a way for slum residents to obtain food, make money, and support their way of life despite all the drawbacks (23). Cattle are frequently kept in small spaces or have their mobility restricted by being tied close to the house. People in slums sell the animal products they obtain to fulfil their needs (24). These cattle provide people with 25 to approximately 40 liters of milk each day. The remainder is consumed in households in addition to being sold in nearby communities through informal marketplaces for

milk and milk products (25). A valuable product that helps ensure food security in slums is milk (26). In Pakistan, the livestock sector is an important aspect of agriculture. Livestock accounts for 37% of the agricultural GDP and about 9% of the overall GDP (27). An estimated 25.5 million buffaloes, 23.8 million cattle, 24.7 million sheep, 54.7 million goats, and 5.4 million other animals make up the livestock population (28). Animals raised for meat, and dairy products get a wide variety of feeds. Animal feeds are frequently contaminated by a variety of factors like environmental pollution and microbial activity (29). It is particularly important to evaluate the risk that pathogenic bacteria present to both animal and human health, in various feeds, and to assess the most effective risk reduction measures, such as the establishment of microbiological standards or criteria within the feed production chain (30). Due to the consumption of the food produced by these animals, feeding is thought to be a crucial aspect in both the health and wellbeing of animals as well as the nutritional intake of humans (31). Managing the available feed supplies effectively is necessary for cattle improvement. The nutritional content of feed for animals is influenced by variables like climate, farming practices, and feed processing methods (32-33). The type of feed given to cattle differs from place to place and according to the socioeconomic status of people, and their livelihood (34). Marketing cattle and cattle products all influences feeding the animals (35). Farm households independently run the majority of Islamabad's milk markets, and they are primarily informal (36). Due to a lack of information on the risks of heavy metals and microbes in milk handling and consumption, the market is subject to a number of limitations, including high risks of microbial contamination and heavy metal contamination (37). Along the dairy value chain, there is insufficient information on the milk handling, quality evaluation, and marketing linkages (38-39). However, because most marketplaces are unregulated, there is a higher risk of microbiological and heavy metal contamination (40). Therefore, this study was conducted to investigate and evaluate the dairy cattle feed and milk for specific contaminants such microorganisms, major and minor elements, and excessive nutrients as well as the risks associated with them along the dairy value chains in H-11/4 slums. The results of this study are expected to be utilized to inform the general public about the health risks linked with milk and feed and potentially implement some realistic solutions to address the problems.

## **Materials and Methods**

### **Study area**

The study area comprised of the un-notified Kachi Abadi of the H-11/4 sector, Islamabad (Figure 1). This informal settlement is home to about 90 families that live in mud houses (41). The majority of the families thrive on a low income sourced from the men working on daily wages. One of the biggest sources of income for these families is harboring animal houses with a minimum of six milk bearing cattle in every one-third home (42). The milk from the cattle is sold out to the nearby I, F and G sectors in the city.

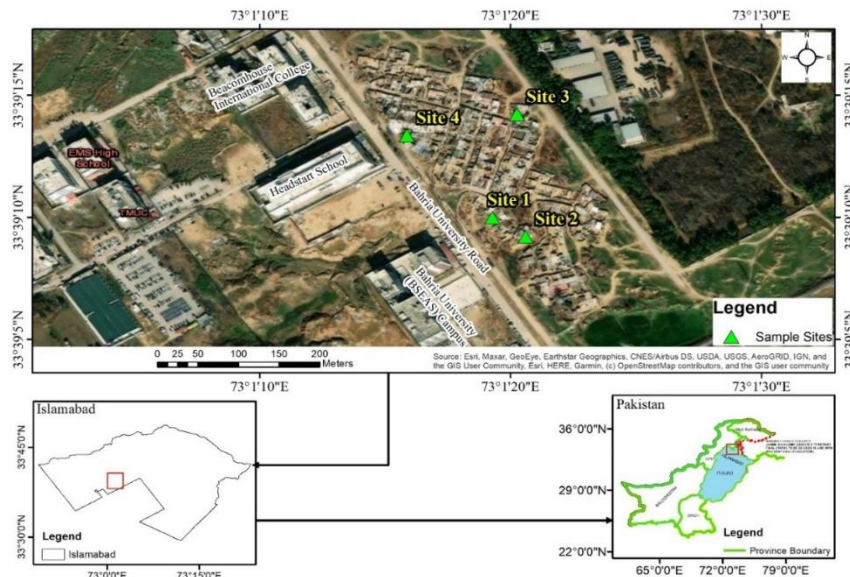


Figure 1: Map of study area showing the sampling sites 1, 2, 3 and 4 respectively

### Sample Collection

Through Random selection, four sampling sites were selected with two dairy farms having some of the greatest number of cattle (fifty to hundreds of cows and buffalos) and the other two from dairy farms having a minimum of five cattle. A total of sixteen samples were collected from each site in sealed and sterilized bottles. Sealed sterilized bottles were used for both milk and feed sample collection. The sealed bottles were only opened during sampling to avoid any external contamination (43-44). Two distinct types of feed were found to be given to the cattle in all four locations. One was wet feed, and the other was dry feed. Similarly, the fresh milk samples were taken at the time of milking in all four sites.



Figure 2: Collection of fresh milk samples from H-11/4 dairy farms Islamabad

### Questionnaire surveys

One of the main objectives of the research was to determine the health impacts of the contaminated milk on the consumers as well as cattle. For this purpose, a questionnaire survey for cattle and consumer health assessment in the slums was conducted.

### Elemental analysis

The analysis for elements in the milk and feed samples was conducted using the inductively coupled plasma-optical emission spectroscopy (Model 5110-ICP-OES). Nitric acid 65% ( $\text{HNO}_3$ )

was used for the digestion of milk and feed samples. 100 ml of milk samples were digested in flasks with 10 ml of concentrated HNO<sub>3</sub>. Wet feed samples underwent the same treatment, while dry feed samples were crushed, diluted with 5 ml of distilled water, and then treated with HNO<sub>3</sub>. All samples were heated at 90-100°C for 10-15 minutes until clear (45-46). After cooling, 30 ml of distilled water was added, and the samples were filtered twice using Whatman filter paper (5, 47). All samples of dry and wet feed, and milk samples were analyzed for major and minor selected elements by using (ICP-OES). Fifteen elements were analyzed, Arsenic (As), Aluminum (Al), Barium (Ba), Copper (Cu), Cobalt (Co), Cadmium (Cd), Chromium (Cr), Lead (Pb), Zinc (Zn), Potassium (K), Selenium (Se), Strontium (Sr), Nickel (Ni), Manganese (Mn), and Molybdenum (Mo).

### Microbial Analysis

The milk and feed sample were evaluated for *Shigella Salmonella* (SS), total bacteria, and total coliforms using SS agar, MacConkey agar, and Nutrient agar. The procedure involved washing bottles with tap water, rinsing with distilled water, and weighing specific quantities of agar powders (48). All materials were autoclaved at 121 °C for 20 minutes to ensure they were free from bacteria. A dilution factor of five was applied to both feed and milk samples by mixing 1ml of each sample with 9ml of sterilized distilled water in test tubes. The diluted solutions were then spread onto petri dishes using a sterilized glass spreader. After sealing and labeling the dishes, they were incubated inverted at 36°C for 24 to 48 hours to prevent moisture accumulation (49). After being in the incubator for 48 hours, the petri dishes were taken out for bacterial enumeration of viable cells (5, 50).

### Determination of nitrates and sulphates

A UV spectrophotometer was used for analysis. Standard solution of sulphate 0.1 mg/ml was taken. The value of absorbance of standard was 0.314. The absorbance of both sample and standard was taken at 425nm. For calculations, the following formula was used.

$$\text{Sulphate mg/L} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 0.1 \times 5 / \text{sample volume} \times 1000 \dots (1)$$

For nitrates testing, digested and filtered samples of feed and milk with HNO<sub>3</sub> were utilized. The formula used for the calculation of nitrates is given by: The absorbance of sample and standard was taken at 220nm. The absorbance of standard was 1.98. The concentration of standard solution (KNO<sub>3</sub>) was 10ppm.

$$\text{Concentration of sample} = \frac{\text{Absorption of sample} \times \text{Concentration of standard}}{\text{Absorption of standard}} (2)$$

### Average daily intake (ADI)

It is expressed as daily dose on per unit body weight basis; it is dose rate which gets averaged over a specific period of exposure time. It is represented as mg/kg/day (51). Formula for the calculation of average daily dose is given by:

$$\text{ADI} = C \times \text{IngR} \times \text{EF} \times \text{ED} / \text{BW} \times \text{AT} \dots (3)$$

Where ADI is average daily intake (mg/kg-day), C is the concentration of contaminant (mg/L, mg/g), IngR is the ingestion rate (L/day, g/day), EF is the exposure frequency (days/year), ED is the exposure duration (years), BW is the body weight (kg) and AT is average time (days/year). The ADD was calculated for the consumers in slums (adults and children) and consumers of sectors I-10, I-11. The average body weight was taken 70kg for adults, and 25kg for children. The average IngR for adults was 0.728 L/day whereas 0.243 L/day for children. The ED was noted during the surveys on the basis of average residency time of the targeted population, the

average value taken was 26 years. The EF taken was 365 days/year. The AT was also calculated, which is the time period over which the exposure gets averaged. It was taken 94910 days/year.

### **Hazard Quotient**

According to the kind and harmful characteristics, many pollutants have diverse impacts on our bodies that are either carcinogenic or noncarcinogenic. A measure used to evaluate the noncarcinogenic effects is the hazard quotient (HQ) (52). It can be calculated using the equation below.

$$HQ = ADI/RfD.... (4)$$

Where RfD stands for the oral reference dose of heavy metals and ADI is average daily intake (mg/kg-day). Oral RfD values (mg/kg-day) for selected fifteen parameters were As 0.0003, Al 1.00, Ba 0.07, Cu 0.04, Co 0.03, Cd 0.001, Cr 0.003, Ni 0.02, Mn 0.14, Mo 0.005, Se 0.005, Sr 0.6, Pb 0.004 and Zn 0.3 (53-54). When the HQ value exceeds 1, it is thought that the body exhibits noncarcinogenic effects. It is assumed to have insignificant noncarcinogenic effects on the human body if it has a value of less than 1 (55).

### **Cancer risk**

To calculate the cancer risk, a unit less probability is used. It may be measured by the following equation as mentioned below:

$$CR=ADI \times CSF_o..... (5)$$

Where ADI is daily average intake (mg/day/Kg), and CSF<sub>o</sub> is the oral cancer slope factor. The CSF<sub>o</sub> is the tool which converts the ADI of contaminant into gradual risk of developing cancer with lifetime exposure (56). For six parameters, As, Cd, Cr, Ni, Pb, and Sr which have been classified as carcinogenic by US EPA, CR was calculated for these six parameters for children and adults. The CSF<sub>o</sub> (mg/kg-day)<sup>-1</sup> for 6 selected carcinogenic parameters were: As 1.50, Cd 0.38, Cr 0.5, Ni 1.7, Sr 8.9E-10, and Pb 0.00085 respectively (57).

### **Statistical analysis**

To calculate the means and standard deviation, for the data IBM SPSS version 25 was used. Correlation was established between concentrations of bacteria and elements in feed and milk using the same software SPSS 25. Pearson correlation was applied to the concentrations of all elements found in dry and wet feed compared with the pollutant concentrations found in milk.

## **Results**

### **Microbial contaminants**

A total of eight milk and eight feed samples from four dairy farms were assessed for microbial contamination by using total plate count (TPC), coliform plate count (CPC), and SS plate count (SSPC) for detection of microbes in milk. The mean CPC was found to be 6.58 (log<sub>10</sub> cfu/ml), mean TPC was 6.25 log<sub>10</sub> cfu/ml, and a mean SS count of 5.04 log<sub>10</sub> cfu/ml. In addition to these, worms and fungus growth in six milk and feed samples were also seen obtained from site 3 and 4, which makes it noticeably clear that the quality of the milk was unsatisfactory and poor (Table 1). All petri dishes showed considerable growth of bacteria on all agars (See Figures 3 a, b, c, and d).

### **Nitrates and Sulphates in milk and feed**

The study also assessed nitrates and sulphates in the collected samples, results showed a mean nitrate of 11.83 mg/L in milk samples, 11.67 mg/L in dry feed and 12.22 mg/L in wet feed samples from dairy farms. Similarly, the mean sulphates in milk samples were 24.3 mg/L, 27.02 mg/L in dry feed and 15.39 mg/L in wet feed samples (Table 2). These values might increase or decrease

depending upon the overall intake of nitrates and sulphates in cattle feed and drinking water source (58).

Table 1: Bacterial concentrations in collected milk and feed samples

Variable	Number of samples	Coliforms (log <sub>10</sub> cfu/ml)	Min (log <sub>10</sub> cfu/ml)	Max (log <sub>10</sub> cfu/ml)
Dry Feed	4	7.59±0.23	7.38	7.89
Wet Feed	4	7.98± 1.49	6	9.44
Milk	4	6.58± 0.39	5.8	7.36
Variable	Number of samples	Total Bacteria (log <sub>10</sub> cfu/ml)	Min (log <sub>10</sub> cfu/ml)	Max (log <sub>10</sub> cfu/ml)
Dry Feed	4	6.56± 4.42	0	9.68
Wet Feed	4	7.89± 0.34	7.47	8.19
Milk	4	6.25± 0.34	5.57	6.93
Variable	Number of samples	SS (log <sub>10</sub> cfu/ml)	Min (log <sub>10</sub> cfu/ml)	Max (log <sub>10</sub> cfu/ml)
Dry Feed	4	3.15± 3.64	0	6.6
Wet Feed	4	1.67± 3.34	0	6.69
Milk	4	5.04± 0.68	3.68	6.4

Table 2: Concentrations of nitrates and sulphates (mg/L) in collected samples

Variables	No of Samples assessed	Sulphates (mg/l)	Minimum	Maximum
Dry Feed	4	27.02±28.38	29.74	83.78
Wet Feed	4	15.39±17.42	19.45	50.23
Milk	4	24.3±28.83	33.3	81.9
Variables	No of Samples assessed	Nitrates (mg/l)	Minimum	Maximum
Dry Feed	4	11.67±11.31	10.95	34.29
Wet Feed	4	12.22±11.84	11.46	35.9
Milk	4	11.83±11.47	11.11	34.77

### Results of surveys

The study used structured questionnaires to gather qualitative data on cattle and consumer health, focusing on management practices, diseases, hygiene, milk production, and transportation. A total of 100 surveys were conducted: 25 in sector I-10, 25 in sector I-11 for consumer health, and 50 in H-11/4 slums for cattle health. Observations on hygiene practices were also recorded, revealing that tube well water was the primary source for drinking and sanitation in dairy farms and households (Figure 4). Water from tube wells was used for milking, cleaning animal sheds, and handwashing, but the containers for milking and storage were difficult to clean. There were no refrigeration facilities in the slums, leading to milk being stored and delivered at room temperature, which encourages bacterial growth. Milking occurred twice daily, with better hygiene practices observed in the morning than in the evening. Hand milking was common, increasing the risk of contamination. Fresh milk was delivered immediately to nearby markets and was also given to calves for nourishment. Farmers who were surveyed had no knowledge related to animal hygiene

and methods for handling livestock and milking. Households typically had one or two lactating animals, while dairy farms had an average three to five. Estimating milk production was challenging due to calves consuming milk before and after milking. More than half of the milk produced was sold, with larger farms producing 20 to 40 kg and smaller ones 10 to 20 kg daily. The survey revealed poor hygiene and handling practices in milk delivery and storage, compromising milk quality. Consumers were uncertain about the sources of cattle and their illnesses, as the qualitative nature of the surveys did not include medical examinations. Respondents reported various diseases affecting cattle, including Q fever, anthrax, chronic respiratory diseases, and lumpy skin disease, leading to symptoms like abortion and mastitis. Many animals suffered miscarriages and deaths, with treatment often sought from costly private veterinarians. Additionally, a significant number of livestock keepers lacked training in animal care and dairy management. (See Tables 5 a, b, and c).

### Elemental analysis

The concentrations of elements in milk from four sites had shown a descending order of **S1** K>Sr>Ba>Se>Al>Mn>Mo>Pb>Ni>Cu>Co>Cd>Cr>Zn, **S2** K>Sr>Ba>Se>Al>Co>Cu>Ni>Cd>Mn>Mo>Cr>Zn>As>Pb, **S3** K>Cu>Sr>Al>Ba>Mn>Se>Pb>Ni>Mo>As>Cd>Co>Cr>Zn and **S4** K>Sr>Ba>Al>Mo>Se>Ni>Co>Cd>Cu>Mn>Cr>Pb>Zn>As. It was clear from the trend that the K, and Sr had the highest levels in all milk samples. Al, Cu, Se, and Ba had also shown slightly increased levels. The levels of elements in dry feed from four sites had shown a descending order of, **S1** K>Sr>Mn>Ba>Se>Al>As>Mo>Co>Cu>Cd>Pb>Ni>Zn>Cr, **S2** K>Al>Sr>Ba>Se>Mn>Cu>Ni>Pb>Mo>Cd>Zn>As>Cr>Co, **S3** K>Sr>Mn>Ba>Zn>Se>Al>Mo>Pb>Co>Cu>Cd>Ni>Cr>As, and **S4** K>Sr>Mn>Ba>Se>Al>Pb>Mo>Co>Cu>Ni>Cd>Zn>Cr>As. The levels of elements in wet feed from four sites had shown a descending order of **S1** K>Sr>Ba>Se>Al>Pb>Mo>Ni>Co>Cu>Cd>Mn>Cr>Zn>As, **S2** K>Sr>Ba>Se>Mo>Al>Pb>Cu>Cd>Co>Mn>Ni>As>Cr>Zn, **S3** K>Ba>Se>Zn>Al>Mo>Co>Cu>Cd>Mn>Sr>Ni>Pb>Cr>As and **S4** K>Sr>Ba>Zn>Al>Se>Mn>Mo>Co>Pb>Ni>Cu>Cd>As>Cr respectively. The dry feed had increased levels of K, Sr, Ba, Mn, Al, and Se. The levels of Zn, K, Sr, Ba, and Al, were highest in wet feed.

### Average daily intake (ADI) of elements

ADI in terms of mg/kg/day was calculated for the children and adults separately by using equation (3) and calculations are shown in the risk assessment Table 6 respectively. ADD was also calculated using microbial and nutrient concentrations (See Tables 3 and 4). Ba levels surpassed the EPA's RfD for both adults and children, with average ADD of  $0.370 \pm 0.094$  mg/kg-day and  $0.359 \pm 0.093$  mg/kg-day, respectively, exceeding the EPA's RfD and the European Food Safety Authority's (EFSA) Tolerable Daily Intake (TDI) of 0.2 mg/kg-day. Milk Cu levels exceeded the WHO's recommendation of 0.2 mg/L, average  $0.250 \pm 0.424$  mg/L, although remained within the EFSA's guideline of 0.3 mg/L. The ADD levels for adults and children surpassed the EPA's RfD and EFSA's TDI, measuring  $2.716 \pm 4.543$  mg/kg-day and  $2.61 \pm 4.37$  mg/kg-day, respectively. Co levels were within WHO and EFSA guidelines, but slightly above the EPA's oral RfD. Adults and children had ADDs of  $0.006 \pm 0.004$  mg/kg-day and  $0.0058 \pm 0.0039$  mg/kg-day, respectively. Cd levels were within WHO and EFSA guidelines (0.003 mg/L) but surpass EPA's RfD and EFSA's TDI, with adults and children reaching ADDs of  $0.00228 \pm 0.00067$  mg/kg-day and  $0.00218 \pm 0.00064$  mg/kg-day, respectively. K levels were within the normal range ( $106.21 \pm 129.49$  mg/L), meeting WHO limits of 35-150 mg/L. Adult and child ADD were within acceptable levels. Milk Mn levels were within acceptable ranges, with values of  $0.004 \pm 0.004$  mg/L, meeting WHO standards of 0.003-0.05 mg/L. Adults and children had ADD below the USDA Tolerable Upper Intake Level (UL). Milk Mo levels were within WHO limits at  $0.00329 \pm 0.00293$  mg/L,

but ADD levels were below the UL for adults and children. The average Se levels were  $0.00812 \pm 0.00195$  mg/L, with ADD of  $0.087 \pm 0.026$  mg/kg-day for adults and  $0.0825 \pm 0.0246$  mg/kg-day for children. Milk Sr levels were within WHO standards, at  $1.123 \pm 0.656$  mg/L. ADD for adults and children was within range. Milk Ni levels averaged  $0.00154$  mg/L, below WHO and EPA standards. WHO's provisional TDI ( $0.005$  mg/kg-bw/day) was surpassed by Adult and Child Daily Doses (EFSA TDI:  $0.03$   $\mu$ g/kg-bw/day). The average Al levels were  $0.08419 \pm 0.12136$  mg/L. The Al ADD levels for adults and children exceeded the JECFA acceptable daily intake of  $0.3$  mg/kg-day, with values of  $0.6416 \pm 1.2729$  and  $0.5919 \pm 1.1924$  mg/kg-day, respectively.

### Hazard quotient (HQ)

The results of HQ for children and adults were acquired by utilizing Equation (4) and are presented in Table 6. The HQ values for As, Cu, Ba, Cd, Mo, Se, Sr, Pb exceeded the prescribed limit of 1. **Site 1:** HQ As>Sr>Se>Mo>Ba>Pb>Cd, **Site 2:** HQ: Se>Sr>Ba>Cd, **Site 3:** HQ: Cu>Se>As>Ba>Pb>Sr>Al>Mo>Cd>Ni, **Site 4:** HQ: Sr>Mo>Se>Ba>Cd>Ni.

### Cancer risk

The consumption of metals from contaminated milk for long-term exposure throughout life is considered to be carcinogenic. According to the US EPA, if cancer risk values lie between  $10^{-6}$  to  $10^{-4}$ , it results in tolerable risk but if it is less than  $10^{-6}$  it can be neglected while, CR values more than  $10^{-4}$  are unacceptable. It has been specified that for all milk samples from four dairy farms only As, Cd and Ni had CR values that surpassed the limit ( $1.0 \times 10^{-4}$ ). **Site 1** had shown this trend: As > Ni > Cd > Pb > Sr, **site 2**, Ni > Cd > Sr, **site 3**, Ni > As > Cd > Cr > Pb, and **site 4** Ni > Cd > Sr. The values of CR for six parameters for children and adults have been computed by utilizing equation 5 and are shown in Table 6.

Table 6: Health risk assessment of selected elements in collected samples of milk and feed on consumers

Elements	Permissible limit mg/L	Value in milk (mg/L)	ADD for Adults mg/kg-day	ADD for Children mg/kg-day	Hazard Quotient (HQ) Adults	Hazard Quotient (HQ) Children	Cancer Risk For Adults	Cancer Risk for Children
As	0.1	$0.0021 \pm 0.003$	$0.0098 \pm 0.014$	$0.0092 \pm 0.013$	<b><math>32.85 \pm 31.97</math></b>	<b><math>15.0 \pm 18.0</math></b>	<b><math>0.0120 \pm 0.017</math></b>	<b><math>0.0115 \pm 0.016</math></b>
Al	0.05	$0.084 \pm 0.12$	$0.642 \pm 1.273$	$0.592 \pm 1.193$	$0.641 \pm 1.273$	$0.592 \pm 1.193$		
Ba	0.7	$0.238 \pm 0.233$	$0.370 \pm 0.094$	$0.359 \pm 0.093$	<b><math>5.52 \pm 1.85</math></b>	<b><math>5.13 \pm 1.76</math></b>		
Cu	0.01	$0.250 \pm 0.424$	$2.716 \pm 4.543$	$2.61 \pm 4.37$	<b><math>89.31 \pm 113.6</math></b>	<b><math>88.03 \pm 112.6</math></b>		
Co	0.005	$0.00059 \pm 0.00044$	$0.006 \pm 0.004$	$0.0058 \pm 0.0039$	$0.21 \pm 0.12$	$0.19 \pm 0.11$		
Cd	0.003	$0.00021 \pm 0.00005$	$0.00228 \pm 0.00067$	$0.00218 \pm 0.00064$	<b><math>2.28 \pm 0.67</math></b>	<b><math>2.18 \pm 0.64</math></b>	<b><math>8.65E-4 \pm 1.63E-4</math></b>	<b><math>8.05E-4 \pm 1.53E-4</math></b>
Cr	0.05	-	-	-	-	-	-	-
K		$106.21 \pm 129.49$	$151.63 \pm 123.59$	$94.03 \pm 49.39$	-	-	-	-
Mn	0.2 - 0.4	$0.004 \pm 0.004$	$0.043 \pm 0.046$	$0.04 \pm 0.043$	$0.46 \pm 0.36$	$0.42 \pm 0.33$	-	-
Mo	0.04	$0.004 \pm 0.0033$	$0.047 \pm 0.035$	$0.045 \pm 0.033$	<b><math>12.87 \pm 7.04</math></b>	<b><math>12.04 \pm 6.59</math></b>		
Se	0.14	$0.00812 \pm 0.0019$	$0.087 \pm 0.026$	$0.0825 \pm 0.025$	<b><math>17.48 \pm 5.27</math></b>	<b><math>16.23 \pm 4.94</math></b>		
Sr	0.5	<b><math>1.123 \pm 0.656</math></b>	$11.89 \pm 7.39$	$11.21 \pm 6.98$	<b><math>20.05 \pm 10.59</math></b>	<b><math>18.77 \pm 9.96</math></b>	$1.25E-8 \pm 4.29E-9$	$1.17E-8 \pm 4.03E-9$
Ni	0.02	$0.00154 \pm 0.00073$	$0.013 \pm 0.007$	$0.0129 \pm 0.007$	$0.675 \pm 0.375$	$0.637 \pm 0.357$	<b><math>2.85 E-3 \pm 1.6E-2</math></b>	<b><math>2.67 E-3 1.5E-2</math></b>
Zn	0.328	-	-	-	-	-		
Pb	0.02	$0.0009 \pm 0.0054$	$0.019 \pm 0.014$	$0.018 \pm 0.013$	<b><math>4.75 \pm 3.51</math></b>	<b><math>4.48 \pm 3.30</math></b>	$1.58E-5 \pm 1.15E-5$	$1.51E-5 \pm 1.1E-5$

### Pearson correlation

The Pearson correlation was applied to the results of bacterial, nutrient, and elemental concentrations in milk samples with the of bacterial, nutrient, and elemental concentrations in dry and wet feed samples to establish the relationship between milk pollutants and feed given. The results of elemental concentrations with dry feed of 3 sites revealed a strong positive relationship ( $r = 0.771-0.987$ ). Similarly for wet feed moderate positive correlation was found ( $r = 0.712-0.859$ ). A moderate correlation was found between bacterial levels with both feeds ( $r = 0.066-0.073$ )

( $r=0.036-0.059$ ) and relatively moderate correlation was found for nutrients levels with both feeds ( $r=0.063-0.083$ ) ( $r=0.051-0.073$ ) (See Table 6). These values suggest a positive direct relationship between the variables indicating that feed given to cattle is maybe the source of contamination, through which the contaminants pass to the animals and then into the milk. They may also be responsible for some diseases in the animals which were seen during surveys potentially causing the health conditions in cattle. The contaminants then pass onto the consumers who consume the milk. The sources of the feed contamination can be attributed to the nearby sources of contamination close to vicinity of dairy farms. It can be from the dust pollution coming from the heavy traffic in the surrounding areas of the slums, harmful discharges from nearby institutes like NESCOM, and PAEC hospital, industrial discharges in I-10 I-9 sectors, high traffic load with vehicular emissions and smoke, all these factors may be contaminating the animal feed and drinking water sources, and therefore the milk. Bacterial contamination could be coming from other sources such as poor processing, handling and storage of milk practices in the dairy farms and unhygienic conditions of cattle in the farms.

Table 7: Results of Pearson correlation between feed and milk contaminant levels

Variable	Dairy farm 1		Dairy farm 2		Dairy farm 3		Dairy farm 4	
	Dry feed	Wet feed	Dry feed	Wet feed	Dry feed	Wet feed	Dry feed	Wet feed
Elements in milk	0.987	0.859	0.963	0.815	0.982	0.771	0.912	0.712
Bactria in milk	0.073	0.059	0.069	0.045	0.072	0.036	0.066	0.036
Nutrients in milk	0.063	0.051	0.073	0.059	0.071	0.061	0.083	0.073

## Discussion

The study aimed at assessing the quality of milk being consumed and sold to nearby hotels, homes and restaurants in four of the dairy farms of H-11/4 slums, Islamabad. The presence of selected biological contaminants (coliforms, total bacteria and *Salmonella Shigella* species), excessive nutrients, sulphates and nitrates, some major and minor elements including some metals were tested within collected milk and feed samples. These analyzed contaminants, microbes, excessive nutrients, and bio accumulative elements can disrupt the body's balance, leading to various health related problems over an extended period of time. Additionally, they can create an environment inside the body that promotes the growth of harmful microorganisms, increasing the risk of foodborne illnesses (59). In light of Pakistan's substantial milk and dairy consumption, illegal dealers may tamper with these products for financial gain. Unfortunately, Pakistan's unstructured and unregulated marketing system results in inadequate consumer milk quality (60). The survey results and direct observation during site visits also revealed that the procedures used in milk collection, storage, transportation, and selling as well as animal housing, feeding, and animal health care all increased the likelihood of microbial contamination due to poor cleanliness and hygiene practices. The majority of samples showed high TPC (6.25 log<sub>10</sub> cfu/ml), SSPC (5.04 log<sub>10</sub> cfu/ml), and CPC (6.58 (log<sub>10</sub> cfu/ml) levels of bacteria, which were higher than the acceptable East Africa Community (EAC), and Food and Drug Administration (FDA) guidelines (61-62). The dairy farms also lie in very close proximity to industrial areas, heavy traffic, and vehicular dust pollution. These may also be the potential reasons the contamination of milk and feed (63). Environmental risk factors play a significant role in the incidence of diseases, both locally and globally. Public health risks have been established as a result of great stability of contaminants in nature and their bioaccumulation in human body (64). Milk contamination is significant in industrial locations, as in the present study region, many factories and industries lie in the premises of the dairy farms, increasing the likelihood of elemental contamination (65). The

study also found a mean nitrate of 11.83 mg/L in milk samples, 11.67 mg/L in dry feed and 12.22 mg/L in wet feed samples. Similarly, the mean sulphates in milk samples were 24.3 mg/L, 27.02 mg/L in dry feed and 15.39 mg/L in wet feed. The examination of milk pollutants indicated high levels of many elements, with Ba levels surpassing the EPA's oral RFD for both adults and children, in addition to high ADD exceeding the EFSA's Tolerable Daily Intake. Mn and Mo levels were below safe limits; however, Se and Sr had high HQ, suggesting potential concerns. Ni levels were within safe limits; however, Al and As, levels exceeded safety thresholds, indicating considerable health risks according to HQ values. Pb HQ levels also exceeded safe limits, suggesting potential health hazards for both adults and children, with Cr values surpassing the US EPA's acceptable risk threshold. The research also administered 100 surveys to evaluate cow and consumer health, uncovering inadequate hygiene standards in dairy management, with tube well water identified as the principal source for sanitation. Milk was kept at ambient temperature, facilitating bacterial proliferation, and numerous farmers were uninformed about appropriate animal hygiene and milking techniques. Numerous infections were documented in cattle, resulting in considerable health complications, while treatment sometimes depended on costly private physicians, underscoring the necessity for enhanced training in animal care and dairy management. The study also found a correlation between elements in milk and feed, suggesting that feed contamination maybe a source of contaminants in milk. The pollutants most likely stem from proximate sources of contamination, such as vehicular emissions and industrial effluents. A positive correlation was also noted between bacterial and nutritional levels, indicating that bacterial contamination may arise from inadequate milk processing, handling, and poor hygiene on the farms. A study by Levkov et al. (2017) found similar concentrations of trace elements (Al, Ba, Cu, Mn, Ni, Sr) in raw milk as observed in the current study (67). Pb levels in the current study were lower than those reported by Al-Rudha et al. (2021), who found levels of 0.62 mg/kg in cow milk (5). Fantuz et al. (2022) also reported comparable levels of macro minerals and trace elements, noting higher levels in buffalo milk (68). Cd and Cr levels were lower in the current study compared to Iqbal et al. (2016) (69). Ba levels were slightly elevated, particularly from site 3. Guler (2007) reported higher levels of several elements, including K, similar to current study (70). Sr levels were higher in the current study than those reported by Guler (2007) and Nabrzyski & Gajewska, (2002) (71). Cu, essential for various bodily functions, was below the IDF's maximum limit at most sites, except for site 3, where it exceeded the safe level. Our results of the study are also very similar to those reported by Al-Ruda et al. (2021) who examined metals and bacteria in cow and buffalo raw milk.

## Conclusion

The present study conducted in the H-11/4 slums of Islamabad, assessed health the risks associated with specific biological and chemical contaminants in dairy cattle feed and milk. Utilizing (TPC), (CPC), and (SSPC), eight milk and eight feed samples from four dairy farms were examined for microbial contamination. All samples revealed significant levels of total coliforms, total bacteria, and SS species. The risk assessment indicated HQ and CR of Ba, Cu, Al, As, and Pb, surpassing safety limits and presenting considerable health risks including potential carcinogenic risks. Although Mn, and Mo, were within acceptable values, increased HQ for Se, and Sr raised more health concerns. High levels of elements like K and Sr in milk pose health risks, especially for children, due to their accumulation in the body. Pollution from traffic and nearby industries may be increasing these contaminants in dairy products. Study also found a significant link between feed and milk elemental contents, suggesting that feed is maybe the primary source of contaminants. A moderate association between bacterial and nutritional levels suggested that contamination may result from poor milk processing and unhygienic farm conditions. The results of surveys revealed inadequate hygiene practices in dairy management, characterized by the use

of tube well water for sanitation and the storage of milk at ambient temperature, which fosters bacterial proliferation. Farmers were uninformed about appropriate animal husbandry, resulting in numerous cattle diseases and dependence on expensive private doctors for treatment. Overall, the milk exhibited signs of poor hygiene and indicates a possible source of milk-borne diseases. To ensure healthy milk, it is recommended to adopt hygienic practices in milking, storage, and transport, and to monitor water and feed quality for cattle.

### **Recommendations**

There is an increased risk of pathogens and foodborne illness in consumers especially for children, infants, pregnant women, older people, and those with weak immune system, consuming milk contaminated with microbes and other contaminants. Therefore, for the health, and safety of consumers, the sale and consumption of healthy and safe milk should be regulated in markets and from dairy farms. This goal must be ensured both at the producer and consumer levels. They need to be educated about the risks associated with milk, especially in slum areas where people do not know much about the health risks, do not have access to better health facilities, and are financially poor. The dairy industry requires sufficient cleaning, including cleaning udders as well as avoiding muddy areas whenever possible. Teats should be cleaned on a regular basis with warm water that contain some disinfectants. These areas especially where milk is sold should be regulated by the concerned authorities. Dairy farmers and people who have kept animals for milk should take care of cleanliness of the animal, and hygiene practices in relation to milk production, handling, storage, and delivery processes to avoid the contamination of milk.

### **Nomenclature**

**ADI** Average Daily Intake.

**CPC** Coliform Plate Count

**CR** Carcinogenic risk

**CRD** Chronic Respiratory Disease

**ED** Exposure duration

**EF** Exposure frequency

**EFSA** European Food Safety Authority

**FAO** Food and Agricultural Organization

**FDA** Food and Drug Administration

**GDP** Gross Domestic Product

**HQ** Hazard Quotient

**IARC** International Agency for Research in Cancer

**NESCOM** National Engineering & Scientific Commission

**PAEC** Pakistan Atomic Energy Commission

**RfD** Oral Reference Dose

**SSPC** Salmonella-Shigella Plate Count

**TPC** Total Plate Count

**USEPA** U.S Environment Protection Authority

### **Conflicts of Interest**

The authors declare no conflict of interest

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## Appendices

Table 3: Average daily intake of Microbes from Milk Consumption

	Microbes	ADI for adults (mg/kg-day)	ADI for children (mg/kg-day)		Microbes	ADI for adults (mg/kg-day)	ADI for children
Site 1	Coliforms	$102.8 \times 10^5$	$96 \times 10^5$	Site 2	Coliforms	$18 \times 10^6$	$168 \times 10^5$
	Total bacteria	$115.7 \times 10^5$	$108 \times 10^5$		Total bacteria	$214.2 \times 10^4$	$20 \times 10^5$
	SS	$26.7 \times 10^4$	$25 \times 10^4$		SS	$267.8 \times 10^4$	$25 \times 10^5$
	Microbes	ADI for adults (mg/kg-day)	ADI for children (mg/kg-day)		Microbes	ADI for adults (mg/kg-day)	ADI for children
Site 3	Coliforms	$182.1 \times 10^4$	$17 \times 10^5$	Site 4	Coliforms	$145.7 \times 10^5$	$136 \times 10^5$
	Total bacteria	$160.7 \times 10^4$	$15 \times 10^5$		Total bacteria	$557.1 \times 10^5$	$52 \times 10^5$
	SS	$42.8 \times 10^3$	$4 \times 10^4$		SS	$396.4 \times 10^3$	$37 \times 10^4$

Table 4: Average daily dose calculated for excessive nutrients

Excessive Nutrients in milk		Site1 (mg/L)	Site2 (mg/L)	Site 3 (mg/L)	Site 4 (mg/L)
ADD for adults (mg/kg-day)		119	124.7	128.5	135
ADD for children (mg/kg-day)	mg/kg-day	111.1	116.4	120	126
ADD for adults (mg/kg-day)		223.9	560.3	163.9	94.1
ADD for children (mg/kg-day)	mg/kg-day	209	523	153	87.8

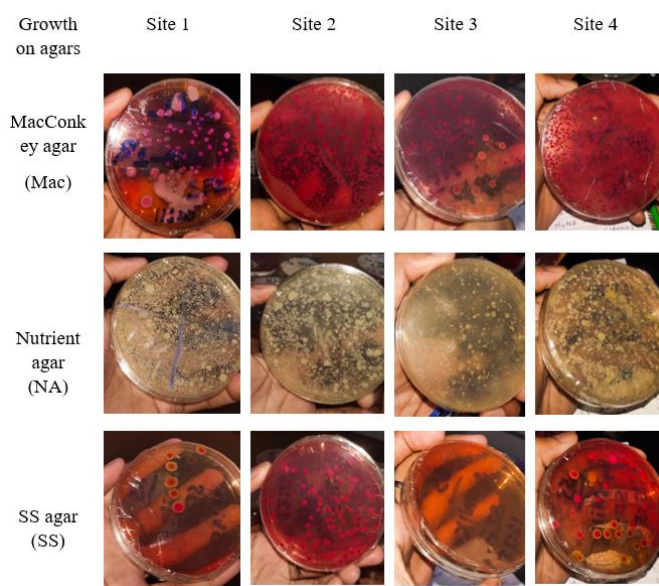


Figure 3(a): Growth of bacteria on milk samples from all four sites

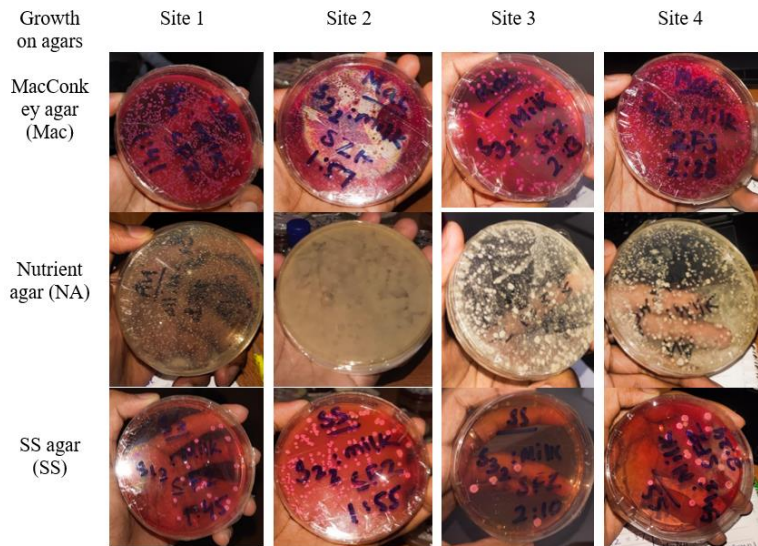


Figure 3(b): Growth of bacteria on milk samples from all four sites

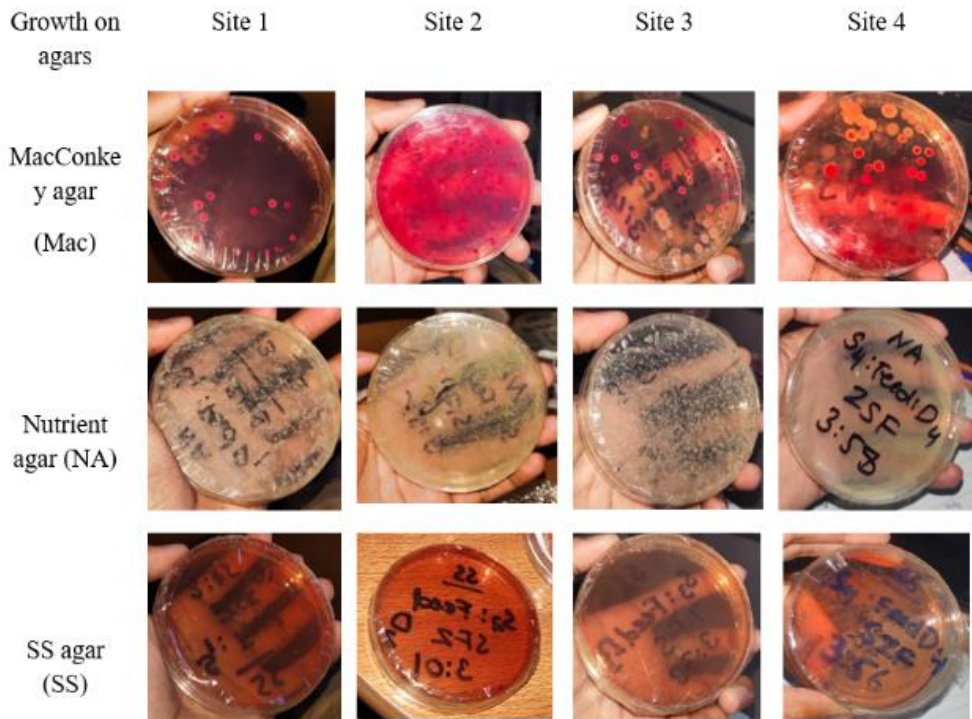


Figure 3(c): Growth of bacteria on dry feed samples from all four sites

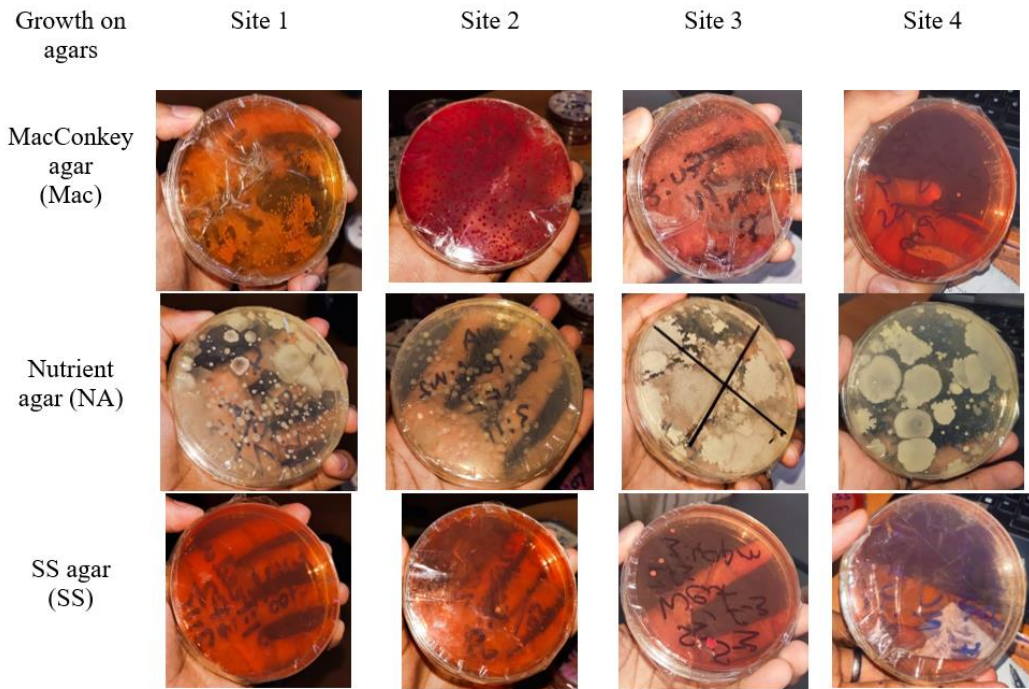


Figure 3(d): Growth of bacteria on wet feed samples from all four sites



Figure 4: Drinking water Containers used in dairy farms for animals and for animal's feed

Table 5a: Animal housing and feeding system as reported by respondents (n = 50)

Variable	Category	No of Respondents (%)
<b>Type of animal house</b>	Trees/boma	45 (90%)
	Block house	5 (5%)
<b>Animal house floor material</b>	Mud/earthen	7 (14%)
	Concrete/cement	43 (86%)
<b>Animal house floor cleaning</b>	Yes	47 (94%)
	No	3 (6%)
<b>Cleaning of animal house floor</b>	Once a day	15 (30%)
	Twice a day	30 (60%)
	Multiple times a day	5 (10%)
<b>Feeding system</b>	Only livestock move	40 (80%)
	Cattle and household moved	5 (10%)
	Stall feeding (Zero Grazing)	42 (84%)
	Herding	8 (16%)

Table 5b: Common practices during milking, storage, and delivery of milk

Variable	Category	No (%) of respondents
<b>Sources of water</b>	Tap water	-
	Tube well (bore-water)	45 (90%)
	Other	5 (10%)
<b>Milking practices</b>	Cleaning animal shed before milking	48 (96%)
	Wash hands before milking	48 (96%)
	Wash cow's teats before milking	48 (96%)
	Wash hands after milking	25 (50%)
<b>Containers used for milk storage</b>	Wide and narrow steel	22 (44%)
	Wide and narrow silver	25 (50%)
	Wide and narrow plastic	3 (6%)
<b>Containers used for delivery/transportation</b>	Wide and narrow steel	24 (48%)
	Wide and narrow plastic	2 (4%)
	Wide narrow silver	24 (48%)
	Plastic bottles /gallons	-
<b>Means of delivery</b>	On foot	35 (70%)
	On bicycle	-
	On motorbike	15 (30%)

Table 5c: Milk Source, sale and storage by milk producers and consumers

Variable	Category	Number of Respondents (%)	
		I-10 n= 25	I-11 n=25
Source of milk	A dairy farm in the same village	7 (28%)	5 (20%)
	More than 2 farms in the same village	9 (36%)	8 (32%)
	A farm from neighboring village	4 (16%)	4 (16%)
	More than 2 farms in the neighboring village	5 (20%)	8 (32%)
Type of milk sold	Raw milk	10 (40%)	10 (40%)
	Boiled milk	15 (60%)	15 (60%)
	Fermented milk	-	-
Customers	Neighboring households	10 (40%)	11 (44%)
	Restaurants/markets/hotels	15 (60%)	14 (56%)
Containers used for milk delivery/selling	Wide necked steel	8 (32%)	5 (20%)
	Wide necked plastic	4 (16%)	4 (16%)
	Narrow necked silver containers	12 (48%)	16 (64%)
How milk is consumed	Boiled	25 (100%)	25 (100%)
	Raw Milk		
Handling/storage of excess milk	Consumed	24 (96%)	23 (92%)
	Reusing next day	1 (4%)	2 (8%)