Human Health Risks Surveillance of Polychlorinated Biphenyls in Bovine Milk from alluvial plain of Punjab, Pakistan

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Abstract

Punjab is the leading province of Pakistan in the production of bovine milk and its consumption. Rapid industrialization, high energy demand and production of waste have increased the risk of PCB toxicity in the environment. This research work was designed to assess human dietary exposure of polychlorinated biphenyls (∑PCBs17 congeners) through ingestion of buffalo and cow's milk from eight main districts of Punjab, Pakistan. The average concentration of ∑DL-PCBs (8.74 ng g^{-1} and 14.60 ng g^{-1}) and ∑I-PCBs (11.54 ng g^{-1} and 18.68 ng g^{-1}) in buffalo and cow milk samples were analyzed respectively. The PCB 156 was predominantly high congener found in both buffalo (2.84 ng g^{-1}) and cow milk (2.86 ng g^{-1}). It was found that the highest PCBs in bovine milk samples were observed in close vicinities of urban and industrial areas. The estimated daily consumptions of DL-PCBs and I-PCBs, from buffalo and cow milk, were below the acceptable daily intake for both adults and children. Moreover, Hazard Quotients (HQ) of ∑PCBs17 congeners value were less than 1.0 in adults and greater in the case of children reflecting the high

chances of cancer risk. Furthermore, comprehensive monitoring for childhood cancer is recommended to establish the relationship in future studies.

Graphical abstract

Key words:

Cattle farming; Dioxin-like PCBs; PCBs accumulation; Dairy Products; Human health risk

1. Introduction

Throughout the world, there is increased concern about the potential health effects of persistent organic pollutants (POPs) owing to their persistence within the environment, long-range transportation, bioaccumulation, and their carcinogenic capacity (Meng et al., 2017; Sohail et al., 2018; Weber et al., 2019). Polychlorinated Biphenyls (PCBs) also termed as chlorinated hydrocarbons (Johnson et al., 1964) are a broad group of organic chemicals produced by human activities like industrialization (Dai et al., 2016) . These are highly toxic compounds which can pose serious health risks to both adults and kids. These were discussed in Stockholm Convention on POPs 2001 because of their potential for adversarial effects on the health of humans and the environment (WHO, 2010). PCBs were produced for their outstanding electric insulation properties and were once extensively used in transformers and capacitors as coolant fluids (Hulin et al., 2020; Kabir et al., 2015). Despite a drastic decline in their manufacture since the 1960s, due to their accessibility, low cost, and adaptability, PCBs are still used for cooling and insulation along with transformer oil, in many developing countries like Pakistan (Eqani et al., 2012; Mahmood et al., 2014a). Furthermore, their use for cable insulation, as plasticizers, pigments, paints, and hydraulic equipment (EPA, 2021) means that there remains a worldwide demand for 4000 MT of PCB/year (Eqani et al., 2012). Direct or indirect production and release of new PCBs is increasing the environmental load and its effect is increasing due to various thermal and industrial processes including incineration, metallurgy and cement production, uncontrolled burning of waste, inappropriate dumping of e-waste, leakage of oil from transformers, open electronics repair workshops, incineration sites, polluted goods, municipal and industrial wastewater disposal (Gong et al., 2017). (Breivik K et al., 2002; EPA, 2004; Eqani et al., 2013; Mahmood et al., 2014a). As such, despite concern about their long term safety and restrictions in their production PCBs are ubiquitous within the environment, PCBs are detectable in various matrices in most countries and human exposure remains possible (Bányiová et al., 2017; Lind et al., 2019).

The 209 PCB congeners are divided into two broad groups: "dioxin-like PCBs (DL-PCBs)" and "indicator PCBs (I-PCBs)" which are often used as markers in pollution studies (Ahmadkhaniha et al., 2017; Rosinska and Karwowska, 2017). There are 12 PCB congeners also called 'Dirty Dozen' whose toxicological effects are comparable to polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (World Health, 2016), because of similarities in properties with PCDDs and PCDFs, these 12 congeners are grouped together and named as dioxin-like PCBs (DL-PCBs) (ATSDR, 2014; Debela and Sheriff, 2021). The indicator PCBs (I-PCBs) are a group of 7 congeners also known as marker PCBs because of their availability and predominance in PCB mixtures (Kim et al., 2004). The congeners of DL-PCBs and I-PCBs are detected in higher concentrations in various environmental matrices including food, human fluids, and tissues (Lyon, 2016), indicating their potential for bioaccumulation and increasing their risk to human health. Concern about environmental levels of PCBs arises as PCBs are categorized as carcinogenic to human beings (Group 1) (IARC, 2012) and it has been estimated that high-fat foods, like dairy products especially milk (Costabeber et al., 2018; Roveda et al., 2006), eggs and animal-based products, contribute 90% of human PCB exposure (EFSA, 2018; Fadaei et al., 2015; FAO/WHO, 2018; Malisch and Kotz, 2014), particularly for infants (Sarode et al., 2016) and children (Lamarche et al., 2016; Larsson et al., 2015). In 2018, 838 million tons of milk were produced globally with a significant contribution coming from India and Pakistan. Currently, Pakistan is the fourth leading milk producer globally (Ishaq et al., 2018; Perisic et al., 2015; Sana et al., 2021) and it's expected that in the coming decade, Pakistan's milk production will continue to increase (FAO, 2019). PCBs levels in milk have been published for many countries including, France (Hulin et al., 2020), Slovakia (Toman et al., 2020), Italy (Bertocchi et al., 2015; Chirollo et al., 2018; Esposito et al., 2010; Tremolada et al., 2014), Brazil (Costabeber et al., 2018; Heck et al., 2007), Iran (Ahmadkhaniha et al., 2017), California (Chen et al., 2017), Mexico (Pérez et al., 2012), Netherland (Baars et al., 2004), Siberia (Mamontova et al., 2007), Belgium (Focant et al., 2003), Germany (Kerst et al., 2004), Chile (Pizarro-Aranguiz et al., 2015), South Korea (Son et al., 2012), India (V.Vanitha et al., 2010) and the United kingdom (Sewart and Jones, 1996). While Reports on PCBs concentrations in other environmental matrices including soil, air, water, and sediments (Ali et al., 2015; Baqar et al., 2017; Eqani et al., 2015; Mahmood et al., 2014a; Syed et al., 2014; Syed et al., 2013), and some elements of the food chain (Mahmood et al., 2014b; Mumtaz et al., 2016) within Pakistan have been published, to date, no reports are available that detail the PCB concentrations in bovine milk and relate them with human health. Acceptable limits of PCBs in milk in Pakistan have also not been defined. The objectives of the current research were, therefore, to explore the concentration levels, homolog and congener distribution of DL-PCBs and I-PCBs in milk from cows and buffalo, to conduct source apportionment and analyze spatial variation in PCB concentrations in milk from various districts, and to evaluate health risks related to PCB consumption in milk by children and adults.

2. Methodology

2.1. Materials

All chemicals used in this study were of grade that is suitable for analysis. PCBs native standards, PCB-209 and Tetra-chloro-meta-xylene (TCmX) were acquired from AccuStandard (America) and stored at -20ºC. Ethanol, Hexane, Acetone and Di-chloro-methane (DCM) were obtained from Merck. Pure N_2 was procured from a local gas filling facility. Columns required for Solid Phase Extraction (SPE), used for cleanup of samples were attained from $SILICYCLE_{Inc}$ (SPEC-R31830B-06P, Certified $SiliaPrep^{M}$ C18, 500 mg/6mL).

2.2. Sampling strategy

Eight districts of Punjab with industrial (Eqani et al., 2015) and agricultural (Ali et al., 2015) significance were selected for the collection of samples (milk) from buffaloes (*n=26*) and cows (*n=28*) (March to December 2018). The study area map is presented as Fig. S1 and the coordinates are given in Table S1. The samples were collected from randomly selected buffaloes and cows, in their native environment, as part of normal milking, during either early morning or evening. Samples were placed in glass bottles of dark color (amber), sealed, labeled, transferred to an icebox, and taken to Environmental Toxicology Laboratory at College of Earth and Environmental Sciences, University of the Punjab, Lahore where they were kept at -20 °C until further analysis (Deti et al., 2014; Ibigbami et al., 2019; Sajid et al., 2016). During the sample collection, a questionnaire (Table S2) was used to record the native environment, living conditions, and the demographic settings of the buffaloes and cows.

2.3. Sample Preparation

Extraction and the cleanup process of PCBs were conducted with minor modifications to previously published methods (Dewan et al., 2013; Sana et al., 2021). Concisely, after maintaining a room temperature of the samples (25 mL per sample), 1 mL was spiked with 50 µL TCmX (100 ppb) (Naqvi et al., 2020; Sohail et al., 2018). Samples were incubated overnight (at 4°C) after the addition of 6 mL of n-hexane and 3 mL of acetone. Samples were then sonicated (with sonicator: Model PS-20A) for 60 minutes on 3°C before being centrifuged (Model 800 Electronic Centrifuge) at 3500 rpm for ten minutes. The resulting supernatant was transferred to a separate glass vial and the residual sample was extracted two times with n-hexane and was added to the same container.

The milk extract was cleaned up by SPE with C18 silica cartridges from SILICYCLE, (Aguilera-Luiz, 2011). Cartridges were primed with n-hexane, before application of samples and elution of PCBs (2x 5 mL of DCM). The eluates were concentrated using pure N_2 gas streaming (Sosan, 2017). Further, 50 µL of 100 ng mL^{-1} strength of ^{13}C-PCB-209 was added to the 1 mL sample (final volume). The samples were filtered through a 0.22 µm filter and kept in 1.5 mL vials (glass) till further analysis.

2.4. Sample Analysis

The PCB content of samples was analyzed using Gas Chromatography-Mass Spectrometer (Agilent Technologies, 5975C inert XL EI/CI MSD using Triple Axis detector; 7890A GC System) tailored along with an AutoSampler (Agilent Technologies 7693), at Environmental Biotechnology Laboratory at University of Glasgow, United Kingdom. Selected Ion Monitoring (SIM) mode was selected used for the study of 17 PCBs (DL-PCBs including PCB 77, 81, 126, 169, 105, 114, 118, 156, 157, 167 and 189 and I-PCBs comprising PCB 28, 52, 101, 138, 153 and 180). A Varian column with specifications (CP-Sil 8CB, 50 m, 0.25 mm, and 0.25 µm) and injector port at 250 °C were used. The basic temperature of the MSD (mass spectrometric detector) was 230 °C and then lowered to 150 °C (quadruple temperature). The succeeding arrangement was used for analyzing all samples: initial 3 minutes temperature was 150 °C then 4 °C per minute up to 290 °C. The isothermal process was maintained for 10 minutes.

2.5. Quality assurance and quality control

Distilled water was used for washing glassware then it was rinsed with DCM and dried at 450 ˚C for almost 6 hours before use. Standards of 1, 10, 20, 50, 100, 200, 500 and 1000 ng g^{-1} were used for developing calibration curves and standardization of instruments. Limit of Detection (LOD) was set at 3x the signal to noise ratio (S/N), while Limit of Quantification (LOQ) was 10x the S/N. The table of LOD and LOQ are given as Table S5. Samples were investigated in small groups with a procedural blank run after every 10 samples. PCB concentration was lesser than the limits in all of the field, procedural and blanks of solvent. The range of the recovery for TCmX was 75-84%. The spiked recovery was 88-151% (mean = 105%). The considered relative standard deviation of the spiked replicates was 20% (mean = 11%). Integration of peaks and data analysis was done by software (Agilent MSD productivity Chemstation).

2.6. DL-PCBs Toxicity Equivalence

The toxicity profile of DL-PCBs was evaluated by assessing the toxicity equivalence (TEQs) by equation (1), where C represents the concentration of DL-PCB congeners and TEF denotes toxic equivalency factor as per the World Health Organization (WHO), International Program on Chemical Safety (WHO-IPCS), 2005 (Van den Berg et al., 2006).

$$TEQ = C \times TEF \text{ --- } (1)$$

2.7. Risk Assessment of Human Health

Guidelines from USEPA were followed for the calculation of health risks (non-carcinogenic and carcinogenic) for adults and children (Dougherty et al., 2000; Sosan, 2017). EDI (ng Kg^{-1} d^{-1}) of PCBs from milk consumption, calculated according to the following formula (equation 2) (Binelli and Provini, 2004).

$$EDI = \frac{CR \times C}{BW} \quad (2)$$

CR is the rate of milk consumption (mL d^{-1}) (Pakistan Economic Survey, 2018), C represents measured concentration (ng g^{-1}) of PCBs congeners, BW is Bodyweight (children = 27.7 Kg and adults = 60 Kg) (Adeleye, 2019; Sosan, 2017). The risk level posed to human beings can be represented by using all these parameters (Dougherty et al., 2000; Wang et al., 2011).

2.7.1. Non-carcinogenic risk assessment

To evaluate the health risks not causing cancer, a comparison of done between EDI (PCBs in milk) and Acceptable Daily Intake (ADI) (EU, 2011a) .

2.7.2. Carcinogenic risk assessment

The Hazard Ratio (HR) was found by following (Dougherty et al., 2000) (equation 3) where CBC (ng Kg^{-1} d^{-1}) is the Cancer Bench Mark ratio which is derived using equation 4.

$$HR = EDI/CBC \quad (3)$$

$$CBC = (RL \times OSF \times BW)/\mathrm{CR} \quad (4)$$

Risk level (RL) is taken as 10^{-6}, Oral Slope Factor (OSF) is measured by unit mg Kg^{-1} d^{-1},

2.8. Data analysis and visualization

Descriptive statistics including mean, standard deviation, range, percentage contribution and distribution frequency were generated for the Milk samples gathered from Punjab districts using Microsoft Excel version 2010. Origin (Pro 8) was used to apply the Krushkal Wallis Test and multivariate statistical analysis of differences in PCBs concentration between study areas. P-value was taken as 0.05. Arc GIS (version 10.2) was used to represent the map of the area under study.

3. Results and Discussion

3.1. PCBs Profile

The concentration profile of DL-PCBs and I-PCBs of the milk samples acquired from buffaloes and cows is given in Table 1. Among all the analyzed milk samples (n = 54) of buffaloes (n = 26) and cows (n = 28), the total means of detected PCB congeners were 20.28 and 33.28 ng g^{-1} respectively.

PCB-156 was the predominant congener among the DL-PCBs for both buffaloes 14.02% and cows 8.59%, followed by PCB-157 (11.50% in buffaloes and 8.21 % in cows). PCB-169 and 126 accounted for 1.20% and 0.73% of the congeners in buffalo's milk samples respectively whereas, PCB-118 and 169 were 7.47% and 4.77% respectively in cows. PCB-189 was not found in investigated milk samples of the cows.

Proportionally PCB-52 and PCB-28 represented 22.12% and 21.96%, respectively, of the I-PCBs in buffalos' milk. In cows, PCB-52 and PCB-28 showed almost equal contribution to the I-PCB load with 23.48% and 22.82% respectively. The percent contribution of PCB-138 to the total I-PCBs for buffaloes and cows' milk was 5.09% and 6.04% respectively. PCB-101 wasn't detected in the samples examined.

The PCB pollution trend of the present study indicates that congeners from group of I-PCBs including PCB-52 and 28 are predominant followed by PCB-156 from the group of DL-PCBs. The reason for highest values of I-PCBs might be sources of these pollutants which include old contaminated buildings (Andersen et al., 2021), sealants used in construction (MECF, 2022), iron and steel making plants (Odabasi et al., 2009b), paints (Hu and Hornbuckle, 2010a), pigments, dyeing and chemical industry, various thermal processes (Lee et al., 2005), waste incineration (Kim and Osako, 2004), e-waste (Fu et al., 2011) and agricultural activities (Mao et al., 2021). Whereas, PCB-156 has been used widely for insulation of various electrical equipment and as plasticizers (Agbo and Abaye, 2016) . Plastic pollution is in itself a big issue in Pakistan (Mukheed M and A, 2020), hence it supports the results of this present study.

3.1.1. Concentration profile of DL-PCBs in Buffaloes and Cow's Milk

Calculation of DL-PCBs profile for the milk samples (buffaloes and cows) indicated that mono ortho congeners (PCB-105, PCB-114, PCB-118, PCB-156, PCB-157, PCB-167 and PCB-189) showed higher values than the non-ortho PCB congeners (PCB-77, PCB-81, PCB-126 and PCB-169). $\sum_{11}$DL-PCBs in buffaloes was 8.74 ng g^{-1} with an average (0.79 ng g^{-1}). Congener with the highest mean concentration was PCB-156 i.e. 2.84 ng g^{-1} (range LOD-20.47 ng $g^{-1)}$. High concentrations of PCB-156 point to the possible use and discharge of commercial PCBs as it's an important component of technical mixtures of Aroclor and Kanechlor (Kim et al., 2009; Malik et al., 2014). It was reported in a study conducted in New York that exposure to Aroclor 1254 was only related to PCB-156 (Seegal et al., 2011). The next highest concentrations of congeners were PCB-157 and PCB-169 with mean concentrations of 2.33 ng g^{-1} and 1.20 ng g^{-1}, respectively. DL-PCB congeners are mainly thought to be produced from industrial activities including coal-burning for sintering iron ore and steel manufacturing. The average concentration of PCB-126 in buffaloes' milk samples is 0.73 ng g^{-1} ranging between LOD-4.11 ng g^{-1}. The potency of PCB-126, however, means that it is often the main contributor (up to 90%) to the toxicity of common PCB mixtures, (Bhavsar et al., 2008; Chirollo et al., 2018; Zhang et al., 2012) and so its presence may have toxicological implications, even though it only made a small contribution in the overall PCB mixtures detected in the samples of the current study. PCB-77 was not detected in any sample. The PCB profile observed in the current study contrast with previous research conducted in Italy (Bertocchi et al., 2015) where PCB-118, PCB-105 and PCB-167 were reported to be present in bovine milk samples at higher concentrations i.e. 3.00 ng g^{-1}, 0.85 ng g^{-1} and 0.21 ng g^{-1} respectively, whereas, PCB-126, PCB-169, PCB-114, PCB-156, PCB-157 and PCB-189 were present in lower concentrations i.e. 0.03, 0.00, 0.07, 0.41, 0.10 and 0.05 ng g^{-1} compared to the present work. Another Italian study conducted in 2010 also reported lower average concentrations of DL-PCBs in bovine milk, except for PCB-118 as compared to current work (Esposito et al., 2010). The study from Chile surveyed for three years, the reported mean values for DL-PCBs were 0.1113, 0.079, and 0.070 ng g^{-1} in each year. All reported PCBs congeners values were also lesser than the mean of buffalo milk samples in this study (Pizarro-Aranguiz et al., 2015). This may be explained by the previous and current exposure of PCBs to various environmental matrices of the area under study (Naqvi et al., 2018; Syed et al., 2013) and calls for action against PCBs.

In cows, the $\sum_{11}$DL-PCBs was 14.60 ng g^{-1}, range LOD-54.23 ng g^{-1}. All analyzed milk samples were predominantly polluted with PCB-156 with the average concentration being 2.86 ng g^{-1}. Congeners with the next highest mean concentrations were PCB-157 and PCB-118 with an average 2.73 ng g^{-1} and 2.49 ng g^{-1}, respectively. Other DL-PCBs which contributed significantly to cows' milk samples were PCB-169, 105, 81, 126, 114, 77 and 167 with mean concentrations 1.59, 1.15, 1.14, 0.92, 0.89, 0.70 and 0.13 ng g^{-1} respectively. The concentration of PCB-126 was detected between LOD-9.47 ng g^{-1} in milk samples of cows. PCB-189 wasn't found in milk samples collected under this study. Comparison of results of the present study with work done in Iran in 2017 indicates that the level of PCBs in the cows' milk in Iran is much higher (Ahmadkhaniha et al., 2017). However, these studies contrast with reports from Slovakia in 2020 where the values of the 7 types of PCBs analyzed were below LOQ. (Toman et al., 2020). The comparison of all congeners in the present study with previous literature for ∑DL-PCBs has been shown in Table S3 so that trends of contamination could be assessed which could provide preliminary data for making remedial plans in future

3.1.2. Concentration Profile of Indicator PCBs in Milk of Buffaloes and Cow

Stockholm Convention for POPs recommended the investigation of 6 I-PCBs (PCB-28, 52, 101, 138, 153 and 180) to characterize the contamination in milk samples (IARC, 2016). None of the samples investigated in this study surpassed the provisional value for the total concentration of I-PCBs, set by the European Union (EU), of 40 ng g^{-1} of raw milk (EU, 2011b). ∑I-PCBs mean concentration in the milk samples of buffaloes is 1.92 ng g^{-1} ranging between 0.00-4.49 ng g^{-1}. Congener profile in buffaloes showed that PCB-52 and PCB-28 were present at the highest average values 4.49 ng g^{-1} and 4.45 ng g^{-1}, respectively with percentage contribution 22.12% and 21.96%. These high values may be indicative of nearby waste dumping sites, agricultural activities, and pigments industries as these are probable main sources of environmental PCB-52 and PCB-28 contamination (Hu and Hornbuckle, 2010b; IARC, 2016). The next highest I-PCB congener concentrations were PCB-153, 138 and 180 with mean concentrations 1.10 ng g^{-1}, 1.03 ng g^{-1} and 0.47 ng g^{-1}. These higher chlorinated PCBs stay in the environment for long durations as they are difficult to degrade, hence they might be considered as indicators of past exposure (Komprda et al., 2019). Manufacturing plants of iron and steel were also reported as potential sources for I-PCBs (Baek et al., 2010). PCB-101 wasn't found in the buffaloes' milk samples of the present study. ∑I-PCBs average in cows was 3.11 ng g^{-1} range LOD-7.81 ng g^{-1}. In the cows' milk samples, PCB-52 showed the highest mean values 7.81 ng g^{-1} tailed by PCB-28 with a mean concentration 7.59 ng g^{-1}. The percent contribution of these congeners was 23.48% and 22.82%, respectively.

PCB-138 and 153 showings mean values 2.01 ng g^{-1} and 1.26 ng g^{-1}, respectively. PCB-101 and PCB-180 weren't detected in the cows' milk samples of the study areas tested in this study. Research work done in California in 2017 presented lower values of I-PCBs when compared with the present study except for PCB-101 which wasn't detected in current work but was found (mean = 0.67 ng g^{-1}) in California. In the California study, of all of the analyzed I-PCBs in the milk samples, PCB-138 PCB-101 and 118 concentrations were the highest (Chen et al., 2017). The differences in I-PCB levels reported in the present study in comparison to previously published literature might be due to differences in season, rainy conditions are known to change PCB levels in soil and fodder crops, also the feeding practices of buffaloes and cows differ greatly between countries and this might have impacted on levels and detection of PCB congeners. Another important factor that could influence the PCB contamination levels in milk is the days in lactation of the buffaloes and cows (Chen et al., 2017; Pérez et al., 2012; Roger Wabeke and Weinstein, 1995). Table S4 shows the current study and previously published literature comparison for I-PCBs.

3.2. Toxic Equivalency of Dioxin-like PCBs

PCB congeners could be characterized concerning their extent of chlorination, substitution tendency, and affinity for binding to receptors. PCBs that show high attraction to aryl hydrocarbon receptor (AhR) is termed as DL-PCBs (Van den Berg et al., 2006). The Toxic Equivalency Factor (TEF) is assigned to congeners after comparing with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) which is extremely noxious among all dioxins, hence a toxic potency 1 i.e. TEF 1 is assigned (Chirollo et al., 2018). The concentration value of each congener was multiplied with its corresponding TEF and resulting TCDD equivalents express toxic equivalents validated through the WHO (Van den Berg et al., 2006). According to regulation (EC) No 1881/2006, milk and other dairy products shouldn't contain more than 0.0055 ng TEQ g^{-1} fat DL-PCBs (Ahmadkhaniha et al., 2017). TEQ values, investigated for DL-PCBs (PCB-77, 81, 105, 114, 118, 126, 156, 157, 167, 169 and 189) Table 2. The sum of ∑DL-PCBs expressed as WHO TEQ_{2005} for buffaloes (0.11 ng g^{-1}) and cows (0.14 ng g^{-1}) sampled for the current study exceeded the recommended maximum. In the milk samples of both buffaloes and cows, PCB 126 has the highest TEQ values i.e. 0.07 ng g^{-1} and 0.09 ng g^{-1} TEQ_{2005}, respectively. PCB 169 has a value at the second-highest level in buffaloes and cows i.e. 0.03 ng g^{-1} and 0.05 ng g^{-1} TEQ_{2005} respectively. These values exceed the given limit of 0.0055 ng g^{-1} by (Regulation, 2011). The PCB TEQ values seen in the current study are higher than previous reports such as 0.00051 ng g^{-1} in Polish milk samples taken from cows (Piskorska-Pliszczynska et al., 2012) and 0.00389 - 0.00595 ng TEQ g^{-1} fat for DL-PCBs in Italian buffaloes milk samples (Chirollo et al., 2018). `

3.3. Spatial Dispersal Patterns and Sources of PCBs in Bovine Milk

The distribution patterns of PCBs in buffaloes and cows' samples from the 8 districts of Punjab, Pakistan included in the current study are depicted in Fig. 1, whereas, percentage contributions of ∑DL-PCBs and ∑I-PCBs in different districts of Punjab are shown in Fig 2 (a and b) respectively**.** The PCBs profiles differed significantly ($p < 0.05$) among the studied districts. Owing to the multiple uses of PCBs as dielectric fluids, plasticizers, flame retardants, adhesives and electric insulation, they may be intentionally manufactured by industries (Perugini et al., 2012). PCBs are emitted into the atmosphere by production, storage and disposal facilities, but they can also leak accidently (Schmid et al., 2003). The released PCBs may deposit and accumulate in the plants, soil and water, which act as natural sinks. The presence of PCBs in water, soil and plants makes these contaminants available for animals to eat when grazing (Esposito et al., 2010).

The highest average ∑PCB concentrations after analyzing all samples from buffalos and cows were observed in Okara district. The investigated high levels of PCBs in the milk of this area might be due to adjacent highway and the industries (cotton, pharmaceutical, marble and granite, plastic, zari, and agro factories) present within 5 Km of the dairy farm sampled (maps, 2021). Being an agricultural area, past usage of PCBs-based pesticides, wood, and solid waste burning practices may also have added to the PCBs level of this site (Naqvi et al., 2020). The second highest values in buffalo contaminated milk were observed in Multan making up 15.44% of the total ΣPCBs concentration. In cows' milk, second place was held by Sialkot making up 18.19% of total ΣPCBs concentrations for cow milk samples in the current study. Lighter PCB homologs (mono to hexa chlorobiphenyls) are linked to few common practices including the burning of agricultural waste, cow dung, and wood. (Balasubramani et al., 2014; Weber et al., 2018).

In milk samples of buffaloes, ∑DL-PCBs were predominant at district Lahore with 21.39% contribution. It might be due to heavy traffic and dense population (Mumtaz et al., 2016). Another study highlighted the adverse PCB contamination in this site especially near industrial and waste dumping areas (Syed et al., 2014). A study conducted on indoor environment of district Lahore also reported high levels of PCBs as compared to other parts of the country (Aslam et al., 2021). It was followed by Multan and Faisalabad with 17.45% and 16.86% contributions. In cows, the highest ∑DL-PCBs were found in Sialkot followed by Gujrat and Okara with the contribution of 21.65%, 21.17% and 20.34% respectively. Many industrial setups are present in the city and surrounding areas of Sialkot district, they might release PCBs into the surrounding environment which could be a reason for high results (Mahmood et al., 2014b). Among I-PCBs (Fig. 2 (b), predominant values were detected at district Okara which was followed by Gujrat by percentages 23.06% and 19.59% in the milk of buffaloes, in the same way, cows' milk also showed predominant values in district Okara tailed by Kasur and Sialkot by percentage contribution 21.08%, 16.68% and 15.49% respectively. A generalized view is that bovines take up PCBs primarily from the feed but there are other known and unknown sources as well which might contribute towards the PCBs levels (McLachlan, 1993). District Multan also contributed significantly with 14.26% and 14.52% of I-PCBs in buffaloes and cows in the province Punjab. This is strengthened by another study, which showed air samples from Multan urban areas with the highest PCB values (Ali et al., 2015). Urban activities in the cities could also be a major source of atmospheric PCB emissions (Ali et al., 2015) and PCBs atmospheric deposition may affect plants and livestock feed greatly (Toman et al., 2020). In the Sahiwal district, within 20 Km distance of the sampled dairy farm, no industrial area or other large-scale commercial activity was identified. Unintentional sources of PCBs emissions including wood and coal combustion (Gullett et al., 2003; Lee et al., 2005), steel plants (Odabasi et al., 2009a), e-waste (Wang et al., 2016), and incineration of domestic solid waste (Kim and Osako, 2004) could be the reason of contamination of the milk samples. The difference between values observed in buffaloes and cows could be due to the variation in food sources and the surrounding environment. Moreover, eating practices of buffaloes and cows differ between locations by their probable impacts on various levels and PCBs exposure. Dumping of residential waste, combustion of waste, electric equipment, PVS, vehicle fuel openly, and other chemical processes may be practiced in the majority part of study areas. PCBs found in human beings greatly depend upon lifestyle and the degree of industrialization. In a study conducted on the Indus River basin, the highest soil PCB concentrations were observed at the agricultural sites (Ali et al., 2015). When the main source of emissions like incinerators, dumpsites and dielectric fluids are not present in the study area (Pérez et al., 2012) then the levels of PCB should fall in permissible limits range. Nevertheless, the current results point towards the existence of other unintended sources and emissions. Thus, it is recommended to maintain surveillance on products used for agriculture and continuous monitoring.

3.4. Health Risk Assessment

3.4.1. Non-Carcinogenic risk

None of the milk samples show EDI exceeding the corresponding ADI limits for both children and adults. For each investigated analyte, the EDI values were higher in children than adults for all milk samples. Among DL-PCBs, PCB-126 showed the highest EDI values 0.72 and 1.57 ng Kg^{-1} d^{-1} (for adults and children) using buffaloes' milk whereas 0.92 and 2.00 ng Kg^{-1} d^{-1} (adults and children) using cows' milk, respectively but lower than ADI 5.5 ng Kg^{-1} throughout this work (Table 3). This high value of PCB-126 may be because of its non-metabolic degradation and these results were also following a study conducted on buffaloes in Italy (Chirollo et al., 2018). ADI of DL-compounds in Dutch people age between 20–25 years, 2.3 and 2.0 pg TEQ Kg^{-1} BW d^{-1} males and females respectively was found by (Patandin, 1999). Two groups of children were studied (1–5 years) and (6 and 10 years), the EDI was higher than in young ones. Similar results were presented by (Wittsiepe et al., 2001) in a similar study conducted in Germany with children 14 to 47 months of age.

No sample in the current study crossed the ADI limits of 40000ng/kg for the I-PCBs under study. PCB-28 and 52 in buffaloes' milk showed EDI values 44.53 & 44.86 ng Kg^{-1} d^{-1} in adult people and 96.45 & 97.17 ng Kg^{-1} d^{-1} in children whereas, cows' milk 75.94 & 78.14 ng Kg^{-1} d^{-1} in adults whereas 164.49 & 169.26 ng Kg^{-1} d^{-1} in children, respectively. PCB-138 showed a value (43.54 ng Kg^{-1} $d^{-}1$) aimed at kids consuming cows' milk (Table 3). PCB-28 are reported to cause developmental neurotoxicity in humans above the ADI (Leijs et al., 2019). In two studies conducted in Brazil on I-PCBs, the EDI value of ∑I-PCBs in raw milk was 1.21 ng Kg^{-1} and in milk powder was found to be 110 ng Kg^{-1}, both results were lower than the present study values for I-PCBs (Costabeber et al., 2018; Heck et al., 2007).

3.4.2. Carcinogenic risk

The potential of PCB contaminated milk to cause cancer is based on cancer benchmark concentration (CBC). Cancer risk, categorized to be one in a million and hazard ratio (HR > 1) is estimated from CBC for analyzing cancer-causing effects in humans (Dougherty et al., 2000). For detailed analysis vulnerable groups especially children should be included in the process of assessment of the risk. The uptake of the pollutants may vary with age. The food and body weight ratio of children is higher than adults so a large amount of DL-PCBs could be ingested. As the children grow up, the dose per unit body weight decreases whereas the consumption per day increases and remains almost the same over 20 years of age (WHO, 2000).

Table 4 represents the results calculated for carcinogenic risk based on the current study. The consumption of milk from different areas of the Punjab province that is contaminated with the ∑DL-PCBs does not pose a cancer threat to adults and kids as the HQ calculated was less than 1. But the results for ∑PCBs including both ∑DL-PCBs and ∑I-PCBs showed a cancer risk for kids in milk samples collected from both buffaloes and cows as the HQ was greater than 1. The HQ values exceeded one for PCBs indicating high risk for infants (Devanathan et al., 2011).

Hence, it could be said that milk from Punjab, Pakistan is safe to use for adults but it may cause risks for children. Previously, carcinogenic risk due to consumption of rice contaminated with PCBs was also reported in Punjab province (Mumtaz et al., 2016). As the significant level of PCBs is reported and detected in Pakistan's environmental matrices, therefore, implementation of educational and awareness activities in the study area might increase the knowledge of local people about the risks and hazards associated with the release of PCBs into the environment, including aspects like major emission sources and how exposure of these could be avoided.

3.5. Conclusion

The current study conducted on buffalos and cows for evaluating the prevalence of DL-PCBs and I-PCBs in their milk and health risk assessment of human beings who consumed the contaminated milk. This research work has not been conducted previously up to the best of our knowledge in the Punjab, Pakistan. Results of the study are also important as they reveal that consumption of milk of Punjab, Pakistan with high levels of DL-PCBs might lead to adverse health effect in children. The current study showed values of ∑DL-PCBs for buffaloes and cows' milk samples to be 0.11 ng g^{-1} and 0.23 ng g^{-1} respectively. These investigated values are higher than the standard 5.5 pg g^{-1} given by the EU commission regulation. Current findings indicate the complexity and regional variability of PCB profiles and sources in bovine milk. The potential non-carcinogenic adverse health effects were calculated and should be emphasized in the sampling areas. Possible cancer risk posed to children is significant. Intentional and unintentional emission of PCBs from industries, burning of wood and coal and poor waste disposal techniques appear to be the main source for PCBs in bovine milk in most sampling areas. The authors recommend continuous monitoring and reduction of PCBs in the environment to minimize exposure.

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Saman Sana: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Roles/ Writing - original draft, Writing - review & editing; **Abdul Qadir:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Resources, Validation, Visualization, Roles/Writing - original draft, Writing - review & editing; **Neil P Evans:** Funding acquisition, Methodology, Resources, Analytical support, Software, Validation, Visualization, Writing - review & editing; **Mehvish Mumtaz**: Investigation, Data curation, Formal analysis, Visualization, review & editing; **Ambreena Mubashir:** Methodology, Data curation, GIS analysis; Maps development, Visualization, review & editing; **Amjad Khan;** Visualization, Sample Collection; Review & editing; **Saif-ur-Rehman Kashif;** Visualization, Methodology validation; Sample Collection; Review & editing; **Habib ur Rehman;** Visualization, Methodology, Validation, review & editing. **Muhammad Zafar Hashmi;** Visualization, Methodology, Validation, review & editing.

Declaration of competing interest & Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Furthermore, the authors declare no conflict of interest

Ethical approval and consent to participate Not applicable

Consent for publication Not applicable

Available of data and materials Datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests, Authors declare that they have no conflict of interests.

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