CALUX Bioassay analysis of PCDD/Fs and dioxin-like PCBs in Bovine milk and Municipal dumping site Ash samples from Indian environment

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Research Article

Keywords: PCDD/Fs, DL-PCBs, Bovine milk, Municipal dump ash, CALUX Assay

Posted Date: April 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-356344/v1>

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Abstract

This study showed the distribution of PCDD/Fs and DL-PCBs in bovine milk and ash samples collected from urban and rural areas of south Tamil Nadu and one milk sample from Japan. Around 25 bovine milk samples and 16 ash samples were collected accordingly. The total TEQ for PCDD/Fs and DL-PCBs were ranged from 0.028 to 7.331 pg TEQ/g fat. Some of the districts were showed higher PCDD/Fs and DL-PCBs concentration in milk and ash samples. Further, BM14 (7.331 pg-TEQ/g fat) and BM21 (6.406 pg-TEQ/g fat) area exceed level of PCDD/Fs and DL-PCBs than WHO regulation limits (6 pg-TEQ/g fat). Likewise, BM3, BM8, and BM14 areas showed higher milk contamination by dioxins, which exceed the WHO regulation limit (3pg-TEQ/g fat).

Similarly, the total TEQ for PCDD/Fs and DL-PCBs in ash samples was ranged from 0.02 to 1.2 ng TEQ/g. AS3 (1.2 ng-TEQ/g) and AS11 (1.06 ng-TEQ/g) areas showed higher dioxins and DL-PCBs among the samples. This study provides an overview of dioxins and dioxin-related compounds contamination in bovine milk and ash samples. Further, the CALUX assay method validation has simplifies the monitoring of dioxin contamination in the environment.

Highlights

- PCDD/Fs and DL-PCBs are the major environmental pollutants released from combustion of waste materials and industries (pesticide and paper manufacturing).
- Bovine milk was contaminated by consumption of contaminated feed and exposed nearby sources (Dioxin producing factories/municipal solid waste dumping sites)
- Bovine milk is the major source for dioxin and dioxin like compounds exposure on humans.
- Ash samples are the sink for accumulate dioxins and related contaminants after combustion or incineration
- Rural areas also polluted by PCDD/Fs and DI-PCBs through dumping of municipal solid wastes.
- Larger cities show exceed level of dioxins and dioxin related compounds than European regulation level (6 pg-TEQ/g fat)

1. Introduction

The PCDD/Fs are also known as endocrine disrupting compounds; consist of 75 polychlorinated dibenzo-*p*-dioxins and 135 polychlorinated dibenzofurans. Similarly, PCBs are a group of 12 polychlorinated biphenyls with similar chemical and toxicological properties of PCDD/Fs. Dioxin like PCBs are carcinogenic and included in group I (Lauby-Secretan et al. 2013). Both of them are chemically inert and resistant to biodegradation, hence persistent in the environment for long period (Esposito et al. 2010).

PCDD/Fs and PCBs are highly lipophilic in nature and can easily accumulate in the fat tissues and bio-magnification. This will leads to severe effects on animals as well as humans and cause adverse effects on environment. PCDD/Fs and PCBs primary source are industrial sectors like metallurgical production,

paper production, pesticide manufacturing, etc (Esposito et al. 2009, Esposito et al. 2010). For example, bovine milk from Northern Italy is highly contaminated by dioxins and PCBs, due to industrialization and agricultural activities (Bertocchi et al. 2015). Humans are mainly exposed through diet, almost 90% of human exposure via food. Some literatures illustrate that, milk and dairy products are significant sources for PCBs and dioxins (27.5–49.6%) in humans (Authority 2012). A higher concentration of dioxins and PCBs had observed in animal origin foodstuffs like milk, meat, fish, and dairy products (Bocio & Domingo 2005, Kiviranta et al. 2004). The becomes a significant concern among researchers to monitor the dioxins and DL-PCBs in environment. Many techniques are available to measure their concentration in environmental matrices.

The conventional methods (high- resolution mass spectrometry (HRMS/HRGC)) applied to measure dioxins in different samples. This method is high accurate and outstanding reliability of separate the specific chemicals. But the disadvantage of the conventional method is time consuming; it takes 4–6 weeks to obtain the concentration of PCDD/Fs and DL-PCBs in samples. Some of the samples cannot be store for long time like pasteurized milk can only store for approximately 7–10 days at 4-7°C.

The reason to choose CALUX assay was cheaper, rapid and can analysis higher number of samples. This method takes only 3–7 days to obtain the results. CALUX assay used to determine the dioxins directly total toxic equivalency (TEQ) and to measure dioxins in different samples, such as eels, mussels, fish, food stuff (beef, cod liver, and milk), human blood plasma, breast milk, soil, and sediment (Warner et al. 2005) (Hoogenboom et al. 2006). Chou et al. (2008) measured PCDD/Fs in cow's milk by CALUX assay.

Dioxins and related chemicals cause effects on the cells through the aryl hydrocarbon receptor, which is a transcription factor. Activation of aryl hydrocarbon receptor (AhR) by dioxins modifies the gene expressions, involved in the cell growth and differentiation (Cancer 1997). Alterations in the gene expression will leads to various toxic effects. The concentration of dioxins and dioxin like compounds in different samples had quantified by chemically activated luciferase gene expression (CALUX) assay. This is an *in vitro* bio-analytical tool applied in research and commercial laboratories. CALUX assay is a reporter gene based cell bioassay; the genetically modified cells used for this assay. The cells that contain the luciferase reporter gene, which response to the dioxin and dioxin-like chemicals. This gene expression was induced by binding and activating of the AhR. The luciferase gene expression depended on the time, dose, AhR dependent and chemical specific manner. The amount of induced luciferease activity can measure the concentration of the chemical. The luminescence by chemical converted to bioassay TEQ value by comparing the response against the given dose of chemical and response to 2, 3, 7, 8- tetrachlorodibenzo-*p*-dioxin (TCDD). CALUX assay used to analyze all AhR active compounds in the extract samples. However, the results interpretation could be complicated compared to chemical analysis because the extracted sample contains a mixture of chemicals (Han et al. 2004). Presence of other contaminants (metals) could affect the CALUX assay readings.

This study's main objective is to determine the dioxins and dioxin like compounds in the Municipal dumping site ash and bovine milk samples using CALUX assay.

2. Materials And Methods

2.1 Chemical reagents and solvents

Hexane (for pesticide residue analysis), RPMI 1640 media, 545 celite, D-PBS and Nonane (for dioxin analysis), Ethyl acetate (for pesticide residue analysis), Acetone, Toluene and sulfuric acid, Sodium sulfate and silica gel (for making column).

2.2 Sample collection

Bovine milk and Municipal dump ash samples of urban and rural areas were collected (January – April, 2012) from the different urban and rural regions (districts) of South India (Fig. 1). 25 milk samples (150 ml of Bovine Milk), and 16 ash samples (50 g of Ash sample) were collected in dumping sites. Milk samples were pack into the screw-type bottle and ash samples covered with zip lock covers.

2.3 Extraction of milk samples

80ml of milk samples were mix with 150ml of acetone in the glass separating funnel. Then the samples were extracted with 40ml of hexane. The samples were settled, and the top layer passed through a 25ml of glass extraction column. Sample and sodium sulfate mixture was packed in an extraction column filled with 20ml of hexane and kept for 10 minutes. Then milk-hexane layer passed into the column. After washing the column, the total solvent has completely evaporated, and then sample vial has been measured for fat content

2.4 Extraction of Ash sample

Two grams of ash samples were weighed, and mixed with 20ml of 2 N of HCl was added. After one hour, HCl-extract was washing with deionized water. The pH of the extract was checked and finally washed with methanol. The obtained HCl extract containing dioxin transferred into the sox let distillation unit. 250 ml of toluene was used for extraction for 16 hours.

Later on, 100 ml of Dichloromethane (DCM) was mixed with 10 ml of sample. The mixture was transferred into the liquid-liquid extraction column and extracted for 10 min for three times. Then the sample was condensed up to 20 ml and further processed. After completing the extraction, the ash sample transferred into 30 ml test tubes. The total 20 ml of ash sample extract, added 4 mL into clean, dry test tubes, and then 20 μ L of nonene was mixed with each ash samples, then kept the samples in multi-solvent extraction unit for 20 mins.

2.5 CALUX Bioassay

The patented dioxin responsive mouse recombinant cell line, H1L6.1c2 was obtained from the Hiyoshi Corporation, Japan (Kumar & Segen 2001) and the method developed by Xenobiotic Detection System (XDS, USA). The cleaned extract to concentrated and then passed through two columns, first with 25 ml of acid silica column and second with 5 ml of active carbon column. 5 ml of the disposable column was

packed with following materials, by glass wool, 0.3 cc of sodium sulfate, 0.7 cc of XCARB (1%), which was an activated carbon patented by XDS (USA) and 0.6 cc of sodium sulfate, rinsed with 5 ml acetone, 10 ml toluene and finally with 10 ml of Hexane. A 10 ml of the disposable column was filled from bottom to top, by glass wool, 1.0 cc sodium sulfate, 4.3 cc of 33% acid silica gel, and 1.0 cc of sodium sulfate and rinsed with 30 ml of Hexane. The acidic silica column placed on top of the carbon column. The fraction containing the Co-PCBs (PCB fraction) eluted with 15 ml ethyl acetate/toluene/hexane (1:1:8) and the second fraction containing PCDD/F (dioxin fraction) was eluted afterwards with 20 ml of toluene. Extracts were concentrated to dryness in a centrifuge under vacuum and reconstituted with 4 ml of Hexane. The samples were stored at 4°C until analysis. Different concentration (lower to a higher level) of 2,3,7,8 – Tetrachloro dibenzo dioxin standard was prepared for the slope of calibrating the CALUX assay (9 point calibration) system (I-Chen Chou et al., 2008). The purified sample extracts in DMSO were suspended in a cell culture medium. Before dosing the plate, hexane solution was transferred into 4 µL of DMSO under vacuum condition in the centrifuge, and finally, 400 µL medium were added to each extract in DMSO. A 2, 3, 7, 8 TCDD standard curves were generated on each plate in addition to the samples. A mixed solution of PCDD/Fs was analyzed for quality control and DMSO alone was studied as blank. This is further serially diluted ranging from 100, 200µl up to 900µl and added to the 9 well plates (STD1 – STD9). After 20–24 h of incubation, cells were examined microscopically for obvious toxicity. Each well rinsed with 50 µL of phosphate-buffered saline (PBS), and 30 µL of cell culture lysis reagent were added to each well then. The plate was shaken for 2 min at room temperature before being placed in the luminometer. After luciferase assay reagent, the light output was integrated and results were expressed in relative light unit (RLU).

3. Mechanism Of Calux Assay

The recombinant cell line used in this assay (H1L6.1c2) was generated by stably transecting the plasmid pGudLuc6.1. This plasmid contains the CYP1A1 dioxin responsive domain. Polychlorinated diaromatic hydrocarbons (PCDH), bind to the AhR. The PCDH-AhR complex then travels to the nucleus of the cell. Activated PCDH-AhR then binds to specific sequences in the DNA called dioxin responsive elements (DRE).

The binding of the PCDH-AhR complex to the DRE will undergo transcription and produce transcripts altered by the founded dioxin. The resulted transcript will direct to luciferase synthesis. The messenger RNA (mRNA) then transfer to the cytoplasm for translation (protein synthesis). New proteins will be synthesized from the polypeptides, called luciferase enzyme. The synthesized protein can cause toxic effects. Dioxin TEQ was measured from luminescence produced by the luciferase reporter gene (Fig. 2). Addition of luciferin (substrate) results in the catabolism by luciferase enzyme, which produces the light measured by luminometer. The produced light was directly proportional to the evoked effects by PCDH.

4. Results And Discussion

4.1 PCDD/Fs and DL-PCBs in bovine milk samples

The concentrations of dioxin and dioxin-like compounds were analyzed by CALUX assay for both bovine milk samples. The concentration of dioxin in milk samples ranged from 0.028 to 7.331 pg TEQ/g fat levels for 25 bovine milk samples, including one sample collected from Japan. Dioxins were contributing almost 83% to the total TEQ concentration. The total concentration of dioxins and DL-PCBs were showed in Table 1. The major source of dioxin exposure on humans was consuming contaminated animal source foods, like milk, egg, meat and fish. The European Union strictly regulated the presence of dioxin and DL-PCBs in food sources to avoid human exposure. In such case, the maximum level of dioxins and DL-PCBs in milk and dairy products was 6.0pg TEQ/g fat. The maximum limit of PCDD/Fs in milk and milk products should be 3 pg TEQ/g fat.

But in our study, the maximum value obtained was 7.331 pg-TEQ/g fat levels, and the overall average level was 3.027 pg-TEQ/g fat, which infers that the values obtained from the current analysis revealed that the dioxin levels were very high compared to the values of Chou et al. (2008). The samples taken from Coimbatore rural, Chennai urban, Erode rural, Salem urban areas were higher than values reported by WHO (EU regulation). PCDD/Fs and DL-PCBs concentration were higher at Cm-R2, among other sampling sites (Fig. 3). These values show an alarming situation to the farmers and consumers. Higher dioxins and DL-PCBs in rural areas may be due to the dumping of municipal wastes, pesticides, etc. Because, municipal wastes had been mostly dumped in the rural areas in most of the districts, which ultimately leads to higher dioxins contamination of rural areas. The present study was compared to dioxins concentration in pasteurized milk. Toxic equivalency of PCDD/Fs for pasteurized milk sample by CALUX assay was ranged between 0.012–0.748 pg – TEQ/g fat levels and the overall average value was 0.246 pg TEQ/g fat (Chou et al. 2008). The reported average value was relatively higher in the present study (1.976 pg TEQ/g) than PCDD/Fs in pasteurized milk samples. One more study reported the dioxins and DL-PCBs in pasteurized bovine milk by Monte Carlo simulation modelling. The mean dioxin and DL-PCBs in pasteurized milk were $0.06 + 0.07$ pg WHO-TEQ/g (Adekunte et al. 2010).

Many kinds of the literature showed the dioxin and DL-PCBs exposure on humans had been attributed by consumption of milk and dairy products. About 38% of the Dutch populations were affected by dioxins and DL-PCBs by consuming contaminated milk and milk products (Sapkota et al. 2007). The reason for dioxins and DL-PCBs in milk could be the feed that cow consuming, which influences the exceeding level than the EU-regulated level. Hoogenboom et al. (2010) reported cows feed (potato peels had kaolinic clay) containing high level of dioxins results in the huge contamination of milk. One study reveals that higher dioxins and DL-PCBs were observed in milk, due to grazing of the cow on contaminated pastures near to factories that produced PCBs (Turrio-Baldassarri et al. 2009). In some cases, cow consumption of roughage could lead to the presence of dioxins and DL-PCBs in milk because roughage was the predominant feed source for dairy cows. Further, ingestion of contaminated soil during grazing will also lead to milk contamination by dioxins (Rychen et al. 2008). Lactation period of cow helps to eliminate the dioxins and DL-PCBs through milk. Early lactation period of cow results in higher elimination of dioxins and DL-PCBs in milk fat, because cow utilizes the stored fat to balance their energy level. Additionally, dioxins and DL-PCBs are lipophilic; hence they accumulate in the body fat. Early lactation period will release the accumulated dioxins because the cows are in negative energy balance (Brambilla et al. 2008).

Milk and dairy products from official control program carried out in Belgium showed a higher level of PCDD/Fs and DL-PCBs determined by HRMS/HRGC; the reported concentration was ranged from 1.58 to 1140.35 pg bio-analytical equivalents (BEQ)/g fat (mean: 31.36 pg BEQ/g fat), respectively (Vromman et al. 2012). Similarly, few works of literature reported the mean concentration of dioxins and DL-PCBs in milk from different countries, like Italy (0.71 pg WHO TEQ/g; DL-PCBs: 1.39 pg WHO TEQ/g) (Esposito et al. 2009, Fattore et al. 2006), China (0.03 pg WHO TEQ/g) (Li et al. 2007), and Belgium (1.57 + 0.71 pg WHO TEQ/g; DL-PCBs: 1.13 pg WHO TEQ/g) (Bilau et al. 2008, Windal et al. 2005). Milk samples from farms located in Brescia City were used to determine PCDD/Fs and DL-PCBs contamination level by high-resolution mass spectrometry (HRMS), and the total WHO-TEQ values were ranged between 1.78–8.16 pg WHO-TEQ/g fat (average: 4.13 pg WHO-TEQ/g fat), respectively. Higher DL-PCBs in the milk may be due to the steel industry, landfills and waste-energy plant and Caffaro factory (produced a higher volume of PCBs) vicinity to study area (Bertocchi et al. 2015). PCDD/Fs and DL-PCBs in milk had been measured from 115 milk collecting plants in France. The concentration range of PCDD/Fs was 0.16–0.76 pg TEQ/g fat (median: 0.47 pg TEQ/g fat) and DL-PCBs concentration was varied between 0.35–1.35 pg TEQ/g fat (median: 0.47 pg TEQ/g fat), respectively. The total concentration of dioxins and DL-PCBs were ranged from 0.60 to 1.77 pg TEQ/g fat (0.78 pg TEQ/g fat). DL-PCBs contribute about 62% to the total TEQ. Consumption of contaminated corn by dairy cows results in a higher level of dioxins and DL-PCBs in milk samples. The source for dioxins could be incineration of waste materials by scrap merchant (Durand et al. 2008).

Bovine milk from different countries showed the undulate contamination level of milk by various sources. Contamination level of PCDD/Fs and DL-PCBs in cow's milk from Austria was 0.14 pg TEQ/g fat and 0.83 pg TEQ/g fat; similarly, milk from German showed higher PCDD/Fs and DL-PCBs concentration (0.45 pg TEQ/g fat and 0.97 pg TEQ/g fat) (Thanner & Moche 2004). Schmid et al. (2003) reported the variation in dioxins and DL-PCBs contamination level based on origins, like a farm from a point source (0.63 + 0.26 pg TEQ/g fat) and remote areas (0.36 + 0.09 pg TEQ/g fat). Milk samples from 63 farms in Italy were collected by personnel of Veterinary Authorities of Regional Sanitary Service (AASSLL) and determined the dioxins and DL-PCBs concentration by HRMS. Total PCDD/Fs and DL-PCBs concentration was ranged between 0.46 and 26.80 pg/g milk fat (mean: 3.06 pg/g milk fat). Some of the milk samples exceed dioxins contamination (above EU regulation limit), close to Napoli and Caserta districts, which contaminate the buffalo herd. Dioxin and DL-PCBs concentration did not exceed the EU regulation limit in the remaining 58 samples (Esposito et al. 2009). Another study also found bovine milk contamination collected from 50 farms in risk areas in 2008–2014 (Italy). The obtained result for dioxins and DL-PCBs ranged as 0.59–2.36 WHO-TEQ pg/g fat, 0.70–1.14 WHO-TEQ pg/g fat and 0.25–2.75 WHO-TEQ pg/g fat for buffalo, bovine and sheep milk, respectively (Serpe et al. 2015). HRMS determined the concentration of dioxins and DL-PCBs in bovine and ovine milk. The sampling areas were close to incineration plant (Valpiana area and Casone area) in Tuscany, which leads to PCDD/Fs and DL-PCBs ranged from 0.71 to 2.9 pg WHO-TEQ/g fat (Ingelido et al. 2009). However, the concentration range was not exceeded the EU regulation (PCDD/Fs: 3 pg WHO-TEQ/g fat and Total TEQs: 6 pg WHO-TEQ/g fat) and action levels (PCDD/Fs and DL-PCBs: 2 pg WHO-TEQ/g fat), respectively (Commission 2006). The

incineration plants were processing municipal solid wastes at a large amount, which was the primary source for dioxins contamination to the surrounding area.

4.2 PCDD/Fs and DL-PCBs in ash samples

The concentration of PCDD/Fs and DL-PCBs in ash samples were ranged from 0.0129–1.208 ng TEQ/g (Table 2). According to WHO values, the results infer that concentration of PCDD/Fs and DL-PCBs were under safety levels (Fig. 4). Total dioxins and DL-PCBs concentration were higher in Erode-1 (1.2 ng-TEQ/g) and Dindigul-1 (1.06 ng-TEQ/g) among other sampling locations. The major source for dioxins and dioxins related compounds the incineration of waste materials carried contamination. Incineration of many organic materials under less than optimal conditions leads to higher dioxins and related compounds release. In such a case, the higher level of dioxins in ash samples from Erode-1 and Dindigul-1 illustrate the huge combustion of organic materials. Municipal solid waste incineration would be the point sources to release PCDD, PCDF and other toxicants; exposed by humans and other organisms (Davoli et al. 2010, Mari et al. 2009, Yang et al. 2006). Particle size also influences the dioxins concentration, fly ash generated from the municipal solid waste collected from major countries showed the dioxins concentration ranged from 0.47 to 25.74 ng I-TEQ/g. Fine ash particles had a higher level of dioxins than larger ash particles (Wu et al. 2016). Fly ash sample collected from sixteen municipal solid waste incinerators (MSWIs) in Taiwan results in 0.78–2.87 ng I-TEQ/g (average: 1.87 ng I-TEQ/g) of dioxins. Further, higher chlorinated PCDDs were highly contributing than lower chlorinated PCDDs (Chang et al. 2011).

Cobo et al. (2009) reported dioxins and DL-PCBs in fly ash samples collected from incineration plant by high-resolution gas chromatography coupled to ion trap low-resolution mass spectrometry (HRGC-QITMS/MS), which processing medical (blood, tissues, body parts, and contaminated sharps) and industrial wastes (polymerization sludge, expired food products, and commercial products). Higher PCDD/Fs (> 185 ng WHO-TEQ/g) and lower DL-PCBs (1.2 ng WHO-EQ/g) concentration were found in the bag filter ash samples. This was corroborating with ESP ash samples (142.1 ng I-TEQ/g) collected from old and small- scale incinerator. Higher PCDD/Fs concentration in fly ash may occur due to old and inefficient operation of wastes (batch process, outdated furnace, slow gas cooling system). Composition of waste that incinerated also play an essential role in the dioxins in ash samples. Different types of ash samples were monitored for dioxins level, in such case types of ash samples from MSWIs showed a different pattern of dioxin concentrations, such as bag filter ash (1.12 and 12.2 ng I-EQ/g), gas scrubber ash (0.26 ng I-TEQ/g), electrostatic precipitators (ESP) (6.7, 8.5 and 142.1 ng I-TEQ/g) and the mixture of semi-dry and bag filter ash samples (0.97–1.5 ng I-EQ/g) (He et al. 2004, Yasuhara & Katami 2007). Dioxin concentration in fly ash may vary depending on the specific operating parameters, like furnace type, capacity, furnace temperature and type of waste (Chang & Chung 1998). Pan et al. (2013) reported the dioxins and DL-PCBs concentration in fly ash collected from 15 municipal concrete waste incinerator plants in different China cities. The concentration of PCDD/Fs and DL-PCBs in fly ash samples were ranged from 0.034 to 2.5 ng- TEQ/g, respectively. This was comparatively lower than dioxins reported in Taiwan (780–2860 ng TEQ/kg) (Chang et al. 2011), Zhejiang (China) (140–2680 ng TEQ/kg) (Chen et al. 2008), and 4 province in (Henan Province, Anhui Province, Jilin Province, Shandong Province and Hubei

Province) China (0.0707–0.7742 ng I-TEQ/g) (Sun et al. 2017). The seasonal variation of dioxin distribution was studied by Sun et al., who discovered the increased dioxins concentration in Hubei Province during winter. Few more studies also found a higher level of dioxins in fly ash from MSWIs in Shanghai, China (7.53, 1.52, 0.98–1.5, 4.16 ng-TEQ/g (Shanghai) and 0.005-87 ng I-TEQ/g (He et al. 2004, Jin et al. 2003, Liu et al. 2015). The PCDD/Fs and DL-PCBs concentration was varied depending upon the particle size of the ash. Ash samples collected from MSWIs in China showed PCDD, PCDF and DL-PCBs concentration ranged from 0.19–2.27 ng I-TEQ/g, 0.2–4.9 ng I-TEQ/g and 0.02–0.22 ng I-TEQ/g, respectively. Dioxins concentration was higher in fine ash particles (diameter 10 – 2.5 and < 2.5) than in the bulk ash (Wu et al. 2016). MSWI in southern Taiwan was studied to observe the dioxins and DL-PCBs level in fly ash. The reported dioxins concentration was ranged from 1.97×10^{-3} to 8.93×10^{-1} ng I-TEQ/g (median: 4.52×10^{-2} ng I-TEQ/g) (Hsieh et al. 2018),], this was corroborated with other countries including Taiwan (laboratory waste: 0.02–1.86 ng I-TEQ/g), and Northeast China (fly ash from MSWI: 3.2-800.1 ng I-TEQ/g), respectively (Li et al. 2016, Wu et al. 2014).

5. Conclusion

The concentration of PCDD/Fs and DL-PCBs was higher in Coimbatore rural and Chennai urban areas than the EU regulation level. This may vary due to consumption of contaminated feed and exposed to PCDD/Fs and DL-PCBs from the municipal dumping site. The samples were collected from cattle near dumping sites, which ultimately influenced the higher dioxin and DL-PCBs concentration in milk. Coimbatore rural, Chennai urban, Salem urban, and Erode rural areas were found higher PCDD/Fs and DL-PCBs concentration in milk. Rural areas had been mostly used for dumping municipal solid waste dumping, which affects the nearby areas. Dioxins are lipophilic; hence it predominantly found in milk fat. In specific, early lactation period will release accumulated dioxins and related compounds at a higher level in milk.

Dioxins and DL-PCBs in ash samples were lower compared to EU regulation level. However, ash samples from Erode-1 and Dindigul-1 showed higher dioxin and DL-PCBs concentration among other sampling sites. Higher organic waste combustion results in a higher level of dioxins and related dioxin compounds in ash samples. Because dioxins are released into the fly ash during combustion, dioxins, and related compounds will affect the surrounding peoples and other species. Hence, further research is needed to better understand the human exposure of dioxins and dioxin related compounds by consuming contaminated milk and exposed to dioxins through inhalation. CALUX assay is a more rapid and cost-effective method that can produce reliable data on dioxins contamination in environmental matrices.

Declarations

Acknowledgement

The authors sincerely thank to HIYOSHI Corporation (Japan), to provide facilities and support for the entire research work. A special thanks to Dr. R. Babu Rajendran (Department of Environmental

Biotechnology, Bharathidasan University) providing this opportunity to accomplish the work in Japan.

Author contribution

Murugasamy Mayilsamy: investigation, data curation, validation and writing - original draft, **Seethappan Sangeetha:** investigation, data curation, validation and review, **Masafumi Nakamura:** method validation and investigation, **Shunkei Ko:** methodology, visualization and investigation, **Muthusamy Govarthan:** visualization, investigation and formal analysis, **Krishnamoorthi Vimalkumar:** writing – review and editing, method validation and supervision.

Funding

This full work carried out and funded by Hiyoshi Corporation, Omihachiman, Shiga prefecture, Japan.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Competing interests: The authors declare no competing interests.

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Location	Sample ID	Fat (%)	Ave.pg fat(Dioxin/Fs)	TEQ /g	Ave.pg fat(PCBs) /g	TEQ /g	Total Dioxin Value
Erode	BM1	3.1858		1.651		0.347	1.998
	BM2	3.2280		2.391		0.645	3.036
	BM3	2.7158		3.885		0.352	4.237
	BM4	5.0354		1.655		0.391	2.046
Salam	BM5	4.1134		1.210		0.502	1.712
	BM6	3.1430		1.262		0.567	1.829
	BM7	3.7956		0.808		0.898	1.706
	BM8	3.4888		4.040		0.433	4.473
Namakkal	BM9	3.6652		1.399		0.632	2.031
	BM10	3.5122		2.587		N.D	2.587
	BM11	4.3930		0.646		0.553	1.199
Coimbatore	BM12	3.7600		0.038		0.648	0.686
	BM13	2.9928		0.028		N.D	0.028
	BM14	3.5580		6.676		0.655	7.331
Tiruppur	BM15	2.0894		0.060		N.D	0.060
	BM16	1.3294		2.191		N.D	2.191
Madurai	BM17	7.3030		1.240		N.D	1.240
	BM18	6.4496		0.099		N.D	0.099
	BM19	0.5588		N.D		2.485	2.485
	BM20	2.2954		2.164		0.632	2.796
Chennai	BM21	0.7158		4.396		2.010	6.406
	BM22	1.5244		N.D		0.172	0.172
Dindigul	BM23	3.4680		2.444		0.044	2.488
	BM24	1.8450		2.614		0.413	3.027
Tiruchirappalli	BM25	4.1426		1.633		0.085	1.718

ND-not detected, U-Urban, R-Rural

Table.2: CALUX Assay result for ash samples

Sample Name	Sample ID	ng TEQ/g (Dioxin/Fs)	ng TEQ/g (PCB)	Total Dioxin Value
Salam	AS1	0.0714	0.0443	0.1157
	AS2	0.0081	0.0048	0.0129
Erode	AS3	1.2009	0.0071	1.208
	AS4	0.4909	0.0057	0.4966
Namakkal	AS5	0.0834	0.0081	0.0915
	AS6	0.1219	0.0039	0.1258
Cuddalore	AS7	0.0032	N.D	0.0032
	AS8	0.0039	0.0167	0.0206
Chennai	AS9	0.1043	0.0119	0.1162
	AS10	0.0892	N.D	0.0892
Dindigul	AS11	1.0637	0.0038	1.0675
	AS12	0.0803	0.0006	0.0809
	AS13	0.0091	0.0122	0.0213
Madurai	AS14	0.2559	N.D	0.2559
	AS15	0.0466	N.D	0.0466
	AS16	0.0593	0.0091	0.0684

ND-not detected

Figures

Figure 1

Bovine milk and Ash samples location of south Indian region

Figure 2

Mechanism of CALUX Assay (Hiyoshi Corporation, Japan), AhR – Aryl hydrocarbon Receptor, ARNT – AhR Nuclear Translocator protein, XRE – Dioxin(X) Responsive Element

Figure 3

Analysis of Total dioxin from bovine milk samples with using CALUX Assay. Concentration levels were measured by pg TEQ/g fat

Figure 4

Analysis of total dioxin from ash samples with using CALUX Assay. Concentration levels were measured by ng TEQ/g

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