CHAPTER V

SUMMARY AND CONCLUSION
V. SUMMARY

The present investigation on “Pesticide Residues in Bovine and Human Milk in Dhanbad City, Dhanbad, Jharkhand” was carried out in the Department of Rural Development and Agricultural Production (RDAP), North Eastern Hill University, Tura Campus, Tura. The collection of bovine and human milk samples and the analytical procedures (extraction and clean up of samples, estimation of pesticide residues) was done within the period of August 2014 to May 2015 which included the three seasons summer, rain and winter.

The samples of cow and buffalo milk were collected from 10 different locations (areas) in Dhanbad city in three different seasons. The human milk samples were also collected from 3 different hospitals and 1 nursing home (that covered the major areas of Dhanbad city). The total number of cow, buffalo and human milk samples collected for all season were 540, 540 and 60 respectively. The milk samples were brought in ice box to the laboratory and were kept in deep-freezers (below 4°C) till the extraction. All the samples were extracted within a period of 24-36 hours. The samples were extracted in the laboratory of the Department of Environmental Science and Engineering of Indian School of Mines (ISM), Dhanbad, Jharkhand. The GC analysis of the extracted samples were also done in the same laboratory. A method described by Faubert Maunder et al (1964), with certain modification by Dhaliwal and Kalra (1977) as described was used for extraction of samples. Clean up of samples was done by acid digestion method as mentioned by Veirov and Ahaaronson (1978), Kapoor et al (1980), Kapoor and Kalra (1988, 1989). The
identification and quantification was carried out in Gas Liquid Chromatograph with peak estimation procedure mentioned earlier. All sets of data were statistically analyzed by using SPSS Version 20 software (SPSS.Inc., Chicago, IL, USA) following statistical designs CRD and FRBD as suggested by Gomez and Gomez, (1984) and Panse and Sukhatme, (1969) respectively. Statistical tools like Analysis of Variance (ANOVA) and correlation were also used for determining the desired objectives.

Pesticide residue levels in bovine and human milk samples

The pesticide residue analysis showed that bovine milk (cow and buffalo) in different locations of Dhanbad city was contaminated with OCP residues of Aldrin, α HCH, β HCH, γ HCH, p,p’-DDE, p,p’-DDD, o,p’-DDT, p,p’-DDT, α Endosulfan, β Endosulfan and Endosulfan Sulfate. Whereas human milk samples were contaminated with OCP residues of α HCH, β HCH, γ HCH, p,p’-DDE, p,p’-DDD, o,p’-DDT, p,p’-DDT and Endosulfan Sulfate.

The level of aldrin concentration (mean) throughout the year in cow and buffalo milk was 0.101896 mg/ml and 0.132343 mg/ml, respectively. High concentration of aldrin in the milk samples reveals the increased improper use of this pesticide for agricultural and commercial purposes although the application of OCPs like aldrin in agriculture have been banned in India. The level of aldrin was comparatively less than the other OCPs detected due to the short half-life of aldrin.

The maximum (0.276111 mg/ml) aldrin concentration in cow milk was found in Bartand area (C) while the minimum (0.005133 mg/ml) aldrin level was found in BankMore area (G). The maximum (0.430539 mg/ml) aldrin concentration in buffalo
milk was found in Jharudih area (D) while the minimum (0.011000 mg/ml) aldrin level was found in BankMore area (G). The above result reveals that highest concentration of aldrin residue persists in the soil, water and air of Bartand (C) and Jharudih (D) making it highly contaminated while BankMore (G) is affected the least by aldrin contamination. The difference in the level of aldrin contamination in different locations of Dhanbad city might possibly be due to difference in the level of pesticide use, fodder supply, breed of cow and buffalo chosen for milk collection and health of animal.

Aldrin was not detected in case of human milk throughout the year. This reveals that the lactating mothers did not encounter the possible sources of aldrin contamination as in cow and buffalo. For example, the maximum percentage of aldrin that entered the cow and buffalo body was from their food sources oat, hay straw as well as oil seeds (Wedberg et al. 1978), which are not the feed of humans. It can also be depicted that minute amount of aldrin residue that enters the human body, is not persistent in the fatty tissue of the body, rather excreted out through urine and faeces, hence not excreted during the time of lactation. This reveals the difference in aldrin metabolism in animal and human body.

The level of $\alpha$ HCH concentration (mean) throughout the year in cow and buffalo milk was 0.028738 mg/ml and 0.044568 mg/ml, respectively. The level of $\beta$ HCH concentration (mean) throughout the year in cow and buffalo milk was 0.104714 mg/ml and 0.158022 mg/ml, respectively. The level of $\gamma$ HCH concentration (mean) throughout the year in cow and buffalo milk was 0.045706 mg/ml and 0.071906 mg/ml, respectively. The level of $\sum$ HCH concentration (mean)
throughout the year in cow and buffalo milk was 0.229639 mg/ml and 0.274496 mg/ml, respectively. High concentration of HCH and its isomers in the milk samples reveals the increased improper use of this pesticide for agricultural (mostly past use) and commercial purposes. The main source of HCH contamination in the milk samples is entry of HCH residue through contaminated feed, drinking contaminated water, breathing contaminated air, contact with contaminated soil. The level of HCH was high in both cow and buffalo milk samples. Also in both, cow and buffalo milk, the highest concentration of OCP residues was that of HCH. From this it can be depicted that being a malaria prone area, the commercial use of HCH has been extensive in and around Dhanbad city. It can also be stated that the persistence level of HCH in bovine milk is higher than other OCP residues like aldrin and endosulfan detected. The presence of various isomers of HCH reveals the rapid metabolic conversion of technical grade HCH into its more persistent and toxic isomers. Among the different isomers of HCH detected, the level of β HCH was higher than α HCH and γ HCH. This reveals that β HCH is highly persistent in nature due to its slow decomposition rate, long half-life and high stability in the environment.

The maximum (0.093883 mg/ml) α HCH concentration in cow milk was found in Wasseypur area (H) while the minimum (0.001422 mg/ml) α HCH level was found in Saraidhela area (A) whereas the maximum (0.164389 mg/ml) α HCH concentration in buffalo milk was found in Bank More area (G) while the minimum (0.002467 mg/ml) α HCH level was found in Saraidhela area (A). The maximum (0.463667 mg/ml) β HCH concentration in cow milk was found in Dhaunsar area (J) while the minimum (0.000111 mg/ml) β HCH level was found in Jharudih area (D) whereas the maximum (0.575167 mg/ml) β HCH concentration in buffalo milk was
found in Dhaunsar area (J) while the minimum (0.001833 mg/ml) β HCH level was found in Bartand area (C). The maximum (0.175206 mg/ml) γ HCH concentration in cow milk was found in Bhuipore area (B) while the minimum (0.004061 mg/ml) γ HCH level was present in Dhaunsar area (J) whereas the maximum (0.212333 mg/ml) γ HCH concentration in buffalo milk was observed in Bhuipore area (B) while the minimum (0.003056 mg/ml) γ HCH level was found to be in Hirapur area (F). The maximum (0.579111 mg/ml) ∑ HCH concentration in cow milk was found in Dhaunsar area (J) while the minimum (0.023889 mg/ml) ∑ HCH level was observed in Saraidhela area (A) whereas the maximum (0.660233 mg/ml) ∑ HCH concentration in buffalo milk was found in Rangatand area (E) while the minimum (0.027556 mg/ml) summation HCH level was present in Hirapur area (F). The above result reveals that Wasseypur (H) and Bank More area (G) has high α HCH residue contamination while Dhaunsar (H) and Bhuipore area (B) has high β HCH and γ HCH contamination, respectively. The irregular and wide spread detection of HCH found in different locations suggests that HCH and its isomers persist in the environment throughout Dhanbad. The difference in the level of α HCH, β HCH and γ HCH contamination in different locations of Dhanbad city might possibly be due difference in the level of use, fodder supply, breed of cow and buffalo chosen for milk collection and health of animal.

The level of α HCH, β HCH and γ HCH concentration (mean) throughout the year in human milk was 0.003319, 0.087468 and 0.037045 mg/ml, respectively. The level of ∑ HCH concentration (mean) throughout the year in human milk was 0.127832 mg/ml. Presence of α HCH in the milk samples reveals the increased improper use of this pesticide for agricultural (mostly past use) and commercial
purposes. The high level of HCH and its isomers finds entry into the human body through contaminated feed, drinking contaminated water, breathing contaminated air, contact with contaminated soil. The level of HCH and its isomers was high in human milk samples, suggesting recent exposure to HCH. Although the use of HCH has reduced in agricultural sector but the extensive use of technical grade HCH as part of Malaria Eradication Program and other disease vector control practice by Health Departments is continuing year after year leading to their spread to the highest trophic level. The presence of various isomers of HCH reveals the rapid metabolic conversion of technical grade HCH into its more persistent and toxic isomers. As seen in case of cow and buffalo milk, among the different isomers of HCH detected, the level of β HCH was higher than α HCH and γ HCH. This reveals that β HCH is highly persistent in nature due to its slow decomposition rate, long half-life and high stability in the environment.

The maximum (0.004507 mg/ml) α HCH concentration in human milk was observed in R C Hazra Memorial Hospital (H4) of Bank More area while the minimum (0.001699 mg/ml) α HCH level was observed in Central Hospital (H1) of Saraidhela area. The maximum (0.278000 mg/ml) β HCH concentration in human milk was observed in Park Clinic Nursing Home (H2) of Hirapur area while the minimum (0.009647 mg/ml) β HCH level was observed in Central Hospital (H1) of Saraidhela area. The maximum (0.079500 mg/ml) γ HCH concentration in human milk was observed in Dwarka Das Jalan Memorial Hospital (H3) of Bartand area while the minimum (0.005547 mg/ml) γ HCH level was observed in Central Hospital (H1) of Saraidhela area. The maximum (0.312447 mg/ml) ∑ HCH concentration in human milk was observed in Park Clinic Nursing Home (H2) of Hirapur area while
the minimum (0.016893 mg/ml) $\sum$ HCH level was observed in Central Hospital (H1) of Saraidhela area. The above result reveals that milk samples of lactating women from R C Hazra Memorial Hospital (H4) of Bank More area showed high $\alpha$ HCH residue contamination while milk samples of lactating women from Park Clinic Nursing Home (H2) of Hirapur area and Dwarka Das Jalan Memorial Hospital (H3) of Bartand area showed high $\beta$ HCH and $\gamma$ HCH contamination, respectively. The irregular and wide spread detection of HCH found in the milk samples of lactating women admitted in hospitals and nursing home of different locations (the woman admitted in a particular hospital, was not necessarily the resident of that particular area) suggests that HCH and its isomers persists in the environment throughout Dhanbad. The difference in the level of $\alpha$ HCH, $\beta$ HCH and $\gamma$ HCH contamination in different locations of Dhanbad city might be due to the difference in food habit and health of the mothers from whom milk had been collected.

The level of p,p'-DDE concentration (mean) throughout the year in cow and buffalo milk was 0.084480 mg/ml and 0.079709 mg/ml, respectively. The level of p,p'-DDD concentration (mean) throughout the year in cow and buffalo milk was 0.012606 mg/ml and 0.022953 mg/ml, respectively. The level of o,p'-DDT concentration (mean) throughout the year in cow and buffalo milk was 0.002151 mg/ml and 0.001988 mg/ml, respectively. The level of p,p'-DDT concentration (mean) throughout the year in cow and buffalo milk was 0.023813 mg/ml and 0.025545 mg/ml, respectively. The level of $\sum$ DDT concentration (mean) throughout the year in cow and buffalo milk was 0.123051 mg/ml and 0.130195 mg/ml, respectively. High concentration of HCH and its isomers in the milk samples reveals the increased improper use of this pesticide for agricultural (mostly past use) and
commercial purposes and its conversion to its metabolites. The reason for the presence of high concentration of DDT and its analogues in the environment is its continued use for the National Malaria Eradication Program (NMEP) of Govt. of India. The main source of HCH contamination in the milk samples is entry of HCH residue through contaminated feed, drinking contaminated water, breathing contaminated air, contact with contaminated soil. The level of DDT was high in both cow and buffalo milk samples. Also in both, cow and buffalo milk, the second highest concentration of OCP residues was that of DDT. Again similar to the case of HCH contamination, it can be depicted that being a malaria prone area, the commercial use of DDT has been extensive in and around Dhanbad city. It can also be stated that the persistence level of DDT in bovine milk is higher than other OCP residues like aldrin and endosulfan detected but less than that of HCH as depicted from the results. The presence of isomers of DDT like p,p’-DDE and p,p’-DDD reveals the rapid metabolic conversion of technical grade DDT into its more persistent and toxic isomers. Among the different isomers and forms of DDT detected, the level of p,p’-DDE was higher than p,p’-DDD, o,p’-DDT and p,p’-DDT. This reveals that p,p’-DDE has high persistence with an estimated half-lives of fifty years in soil. The relatively long half-life of p,p’-DDE in animals explains its tendency to undergo strong biomagnification with movement along food chains. In contrast, the relatively lower concentration of p,p’-DDT is due its rapid metabolic conversion due to its less persistence than the other forms of DDT.

The maximum (0.383389 mg/ml) p,p’-DDE concentration in cow milk was present in Jharudih area (D) while the minimum (0.004283 mg/ml) p,p’-DDE level was present in Rangatand area (E) whereas the maximum (0.313056 mg/ml) p,p’-
DDE concentration in buffalo milk was present in Jharudih area (D) while the minimum (0.012572 mg/ml) p,p’-DDE level was present in Bhuipore area (B). The maximum (0.027167 mg/ml) p,p’-DDD concentration in cow milk was present in Jharudih area (D) while the minimum (0.000978 mg/ml) p,p’-DDD level was present in Rangatand area (E) whereas the maximum (0.075333 mg/ml) p,p’-DDD concentration in buffalo milk was found in Matkuriya area (I) while the minimum (0.001522 mg/ml) p,p’-DDD level was found in Rangatand area (E). The maximum (0.003483 mg/ml) o,p’-DDT concentration in cow milk was found in Bhuipore area (B) while the minimum (0.000389 mg/ml) o,p’-DDT level was found in Wasseypur area (H) whereas the maximum (0.006833 mg/ml) o,p’-DDT concentration in buffalo milk was found in Jharudih area (D) while the minimum (0.000367 mg/ml) o,p’-DDT level was found in Saraidhela area (A). The maximum (0.074889 mg/ml) p,p’-DDT concentration in cow milk was in Bhuipore area (B) while the minimum (0.001556 mg/ml) p,p’-DDT level was found in Matkuriya area (I) whereas the maximum (0.071222 mg/ml) p,p’-DDT concentration in buffalo milk was found in Bhuipore area (B) while the minimum (0.000556 mg/ml) p,p’-DDT level was found in Rangatand area (E). The maximum (0.438389 mg/ml) summation DDT concentration in cow milk was observed in Jharudih area (D) while the minimum (0.022094 mg/ml) summation DDT level was observed in Rangatand area (E) whereas the maximum (0.376222 mg/ml) summation DDT concentration in buffalo milk was found in Jharudih area (D) while the minimum (0.017600 mg/ml) summation DDT level was found in Rangatand area (E). The above result reveals that area Jharudih (D) has high p,p’-DDE residue contamination while area Jharudih (D) and Matkuriya (I) has high p,p’-DDD contamination. High o,p’-DDT
contamination was found in Bhuipore (B) and Jharudih area (D). High p,p’-DDT contamination was found in Bhuipore area (B). This shows Bhuipore (B) and Jharudih (D) area of Dhanbad city was highly contamination with DDT residue and its metabolites. From the above result, it can also be stated that Rangatand (E) area was having lower contamination of DDT and its metabolites. The difference in the level of p,p’-DDE, p,p’-DDD, o,p’-DDT and p,p’-DDT contamination in different locations of Dhanbad city might possibly be due to difference in the level of pesticide use, fodder supply, breed of cow and buffalo chosen for milk collection and health of animal.

The level of p,p’-DDE, p,p’-DDD, o,p’-DDT and p,p’-DDT concentration (mean) throughout the year in human milk was found to be 0.992252, 0.025245, 0.000468 and 1.024617 mg/ml respectively. The level of $\Sigma$ DDT concentration (mean) throughout the year in human milk was found to be 2.042582 mg/ml. Presence of DDT in the milk samples reveals the increased improper use of this pesticide for agricultural (mostly past use) and commercial purposes in Dhanbad city. The high level of HCH and its isomers finds entry into the human body through contaminated fruits, vegetables, meat, milk and milk products, etc. feed, drinking contaminated water, breathing contaminated air, contact with contaminated soil. The level of DDT and its isomers was high in human milk samples, suggesting recent exposure to DDT. Although the use of DDT has reduced in agricultural sector but the extensive use of technical grade DDT as part of Malaria Eradication Program and other disease vector control practice by Health Departments is continuing year after year leading to their spread to the highest trophic level. The presence of various isomers of DDT reveals the rapid metabolic conversion of technical grade HCH into
its more persistent and toxic isomers. In contrast to the case in cow and buffalo milk, among the different isomers of DDT detected, the level of p,p’-DDT was highest. This reveals that the metabolism of DDT in the body of human is different from that in the animal body. Once DDT enters human body, high metabolic conversion takes place.

The maximum (1.392867 mg/ml) p,p’-DDE concentration in human milk was observed in Park Clinic Nursing Home (H2) of Hirapur area while the minimum (0.438800 mg/ml) p,p’-DDE level was observed in Central Hospital (H1) of Saraidhela area. The maximum (0.038548 mg/ml) p,p’-DDD concentration in human milk was observed in R C Hazra Memorial Hospital (H4) of Bank More area while the minimum (0.009799 mg/ml) p,p’-DDD level was observed in Central Hospital (H1) of Saraidhela area. The maximum (0.000733 mg/ml) o,p’-DDT concentration in human milk was observed in R C Hazra Memorial Hospital (H4) of Bank More area while the minimum (0.000133 mg/ml) o,p’-DDT level was observed in Central Hospital (H1) of Saraidhela area. The maximum (1.463600 mg/ml) p,p’-DDT concentration in human milk was observed in R C Hazra Memorial Hospital (H4) of Bank More area while the minimum (0.393000 mg/ml) p,p’-DDT level was observed in Central Hospital (H1) of Saraidhela area. The maximum (2.835067 mg/ml) summation DDT concentration in human milk was observed in Park Clinic Nursing Home (H2) of Hirapur area while the minimum (0.841732 mg/ml) summation DDT level was observed in Central Hospital (H1) of Saraidhela area. The above result reveals that milk samples of lactating women from Park Clinic Nursing Home (H2) of Hirapur area showed high p,p’-DDE residue contamination while milk samples of lactating women from R C Hazra Memorial Hospital (H4) of Bank More area
showed high p,p’-DDD, o,p’-DDT and p,p’-DDT contamination. As the women admitted in a particular hospital, was not necessarily the resident of that particular area, it cannot be ascertained that Bank More area people has high DDT contamination in their body. And hence it can be suggested that DDT and its isomers persists in the environment throughout Dhanbad. The difference in the level of p,p’-DDE, p,p’-DDD, o,p’-DDT and p,p’-DDT contamination in different locations of Dhanbad city might be due to the difference in food habit and health of the mothers from whom milk had been collected.

The level of α-Endosulfan concentration (mean) throughout the year in cow and buffalo milk was 0.001944 mg/ml and 0.003794 mg/ml, respectively. The level of β-Endosulfan concentration (mean) throughout the year in cow and buffalo milk was 0.002301 mg/ml and 0.003207 mg/ml, respectively. The level of Endosulfan Sulfate concentration (mean) throughout the year in cow and buffalo milk was 0.000307 mg/ml and 0.000504 mg/ml, respectively. The level of Σ Endosulfan concentration (mean) throughout the year in cow and buffalo milk was 0.004552 mg/ml and 0.007506 mg/ml, respectively. The presence of detectable amount of Endosulfan isomers revealed the use of endosulfan by the farmers for agricultural and commercial purposes (John P J et al 2001). Endosulfan and its isomers may find their way into body of cattle through the contaminated food, water and air or through skin contact as endosulfan is sprayed by the health department for treating malaria, filariasis, dengue, cholera, louse borne typhus control, termites and the indoor spraying on the walls and roofs. The level of endosulfan isomers was low in both cow and buffalo milk samples. It can also be stated that the persistence level of endosulfan and its isomers in bovine milk is lower than other OCP residues like
The maximum (0.006833 mg/ml) α-Endosulfan concentration in cow milk was found to be in area Bhuipore (B) and the minimum (0.000278 mg/ml) α-Endosulfan level was found to be in area Hirapur (F) while the maximum (0.013278 mg/ml) α-Endosulfan concentration in buffalo milk was found to be in area Bhuipore (B) and the minimum (0.000222 mg/ml) α-Endosulfan level was found to be in area Hirapur (F). The maximum (0.007778 mg/ml) β-Endosulfan concentration in cow milk was found to be in area Bhuipore (B) and the minimum (0.000167 mg/ml) β-Endosulfan level was found to be in area Wasseypur (H) and area Matkuriya (I) while the maximum (0.014500 mg/ml) β-Endosulfan concentration in buffalo milk was found to be in area Jharudih (D) and the minimum (0.000278 mg/ml) β-Endosulfan level was found to be in area Wasseypur (H). The maximum (0.000783 mg/ml) Endosulfan Sulfate concentration in cow milk was found to be in area Wasseypur (H) and the minimum (0 mg/ml) Endosulfan Sulfate level was found to be in area Bartand (C), Hirapur (F) and Matkuriya (I) while the maximum (0.001389 mg/ml) Endosulfan Sulfate concentration in buffalo milk was found to be in area Bhuipore (B) and the minimum (0.000111 mg/ml) Endosulfan Sulfate level was found to be in area Hirapur (F). The maximum (0.015 mg/ml) Σ Endosulfan concentration in cow milk was found to be in area Bhuipore (B) and the minimum (0.000333 mg/ml) Σ Endosulfan level was found to be in area Hirapur (F) while the
maximum (0.021622 mg/ml) $\sum$ Endosulfan concentration in buffalo milk was found to be in area Jharudih (D) and the minimum (0.001056 mg/ml) $\sum$ Endosulfan level was found to be in area Hirapur (F). The above result reveals that area Bhuipore (B) has high $\alpha$ HCH residue contamination while area Bhuipore (B), Jharudih (D) and area Bhuipore (B), Wasseypur (H) has high $\beta$-Endosulfan and Endosulfan Sulfate contamination, respectively. From the above result, it can be depicted that highest contamination of endosulfan and its isomers is in Bhuipore (B) area. The difference in the level of $\alpha$-Endosulfan, $\beta$-Endosulfan and Endosulfan Sulfate contamination in different locations of Dhanbad city might possibly be due to difference in the level of pesticide use, fodder supply, breed of cow and buffalo chosen for milk collection and health of animal.

$\alpha$-Endosulfan and $\beta$-Endosulfan was not detected in case of human milk in any of the places of study throughout the year. This may be due to the fact that the maximum percentage of Endosulfan that entered the cow and buffalo body was from their food sources oat, hay straw as well as oil seeds (Wedberg et al 1978), which are not the feed of humans. As endosulfan use for agricultural purposes is less in and around Dhanbad city, the level of endosulfan and its isomers even the cattle feed are quite less, thus not reaching the human body in significant amount. As $\alpha$-Endosulfan and $\beta$-Endosulfan can leave the body through urine just a few days after exposure, so the small amount that enters human body rapidly gets eliminated. This also reveals the difference in aldrin metabolism in animal and human body. The level of Endosulfan Sulfate concentration (mean) throughout the year in human milk was found to be 0.000072 mg/ml, respectively. The level of $\sum$ Endosulfan concentration (mean) throughout the year in human milk was found to be 0.000072 mg/ml. The
presence of endosulfan sulfate in human milk suggested its higher persistent level in comparison to the other isomers of endosulfan.

The maximum (0.000113 mg/ml) Endosulfan Sulfate concentration in human milk was found to be in Park Clinic Nursing Home (H2) of Hirapur area and the minimum (0.000020 mg/ml) Endosulfan Sulfate level was found to be in R C Hazra Memorial Hospital (H4) of Bank More area. The maximum (0.000113 mg/ml) \( \sum \) Endosulfan concentration in human milk was found to be in Park Clinic Nursing Home (H2) of Hirapur area and the minimum (0.000020 mg/ml) \( \sum \) Endosulfan level was found to be in R C Hazra Memorial Hospital (H4) of Bank More area. The above result reveals that milk samples of lactating women from Park Clinic Nursing Home (H2) of Hirapur area showed high Endosulfan Sulfate residue contamination. As the women admitted in a particular hospital, was not necessarily the resident of that particular area, it cannot be ascertained that Bank More area people has high DDT contamination in their body. The difference in the level of Endosulfan Sulfate contamination in different locations of Dhanbad city might be due to the difference in food habit and health of the mothers from whom milk had been collected.

The level of aldrin, HCH, DDT and endosulfan concentration throughout the year was found to be 0.101896, 0.229639, 0.123051 and 0.004552 mg/ml, respectively in cow milk whereas the level of aldrin, HCH, DDT and endosulfan concentration throughout the year was found to be 0.132343, 0.274496, 0.130195 and 0.007506 mg/ml, respectively in buffalo milk. From the above data, it can be analyzed that the highest amount of OCP residues was found in buffalo milk as compared to that in cow milk. The reason for higher OCP residue levels in buffalo
milk than cow milk due to the high fat content (7.47%) of the buffalo milk as compared to cow milk (3.66%), as these OCPs concentrate in the fatty tissues of the body. Another significant reason for higher concentration of OCP residues in buffalo milk than cow milk is due to difference in dietary habit of buffalo, like different fodder and more diet than the cows.

**Seasonal variation in the pesticide residue level in bovine and human milk**

The mean aldrin concentration in cow milk was higher (0.1652883 mg/ml) in the winter months compared to summer (0.098732 mg/ml) and rainy (0.041668 mg/ml) and the data obtained for difference in aldrin concentration in three seasons was found to be significant (P value= 0.049; P ≤ 0.05), which means there is a significant difference in the level of aldrin in the milk samples in three seasons. The significant seasonal variation was due to the significant (P value = 0.014; P ≤ 0.05) difference in the mean aldrin concentration during winter and rainy season. The mean aldrin concentration in buffalo milk was higher (0.233166 mg/ml) in the winter months compared to summer (0.110582 mg/ml) and rainy (0.053280 mg/ml). The data obtained for difference in aldrin concentration in three seasons was found to be highly significant (P value= 0.001; P ≤ 0.01), which means there is a highly significant level of difference in the level of aldrin in milk samples in three seasons. The possible reason for this trend might be that the influences of heat, wind and rain, which are the characteristics of the summer and rainy seasons, are very low during winter seasons so that the dissipation of pesticide residues does not take place very rapidly. This reveals that there is significant effect of seasonal factors on the level of aldrin accumulation in the milk samples in Dhanbad city.
The difference in aldrin concentration in different locations in cow and buffalo milk was found highly significant (P value= 0.000; P ≤ 0.01), which means there is a highly significant level of difference in the level of aldrin both in cow and buffalo milk in the different locations. The possible reason may be possibly due to difference in the level of pesticide use, fodder supply, breed of cow and buffalo chosen for milk collection and health of animal.

In case of human milk, aldrin residue was not detected at all places of the study area throughout the year.

The mean α HCH concentration in cow milk was higher (0.048873 mg/ml) in the rainy months compared to summer (0.022462 mg/ml) and winter (0.01488 mg/ml) and data obtained for difference in α HCH concentration in three seasons was found to be highly significant (P value= 0.000; P ≤ 0.01), which means there is a highly significant difference in the level of α HCH in the three seasons. The significant seasonal variation was due to the highly significant difference of α HCH concentration during rainy and summer season (P value = 0.004; P ≤ 0.01) and during rainy and winter season (P value = 0.000; P ≤0.01).

The mean α HCH concentration in buffalo milk was higher (0.080467 mg/ml) in the rainy months compared to summer (0.037110 mg/ml) and winter (0.016128 mg/ml). The data obtained for difference in α HCH concentration in three seasons was found highly significant (P value= 0.000; P ≤ 0.01), which means there is a highly significant level of difference in the level of α HCH in the milk samples in three seasons. The significant seasonal variation was due to the highly significant difference of α HCH concentration during rainy and summer season (P value =
0.007; P ≤ 0.01) and during rainy and winter season (P value = 0.000; P ≤ 0.01). The possible reason for this trend might be that the influences of heat, wind and rain, which are the characteristics of the summer and rainy seasons, are very low during winter seasons so that the dissipation of pesticide residues does not take place very rapidly. This reveals that there is significant effect of seasonal factors on the level of α HCH accumulation in the milk samples in Dhanbad city.

The difference in α HCH concentration in different locations in cow and buffalo milk was found highly significant (P value= 0.000; P ≤ 0.01), which means there is a highly significant level of difference in the level of α HCH both in cow and buffalo milk in the different locations. The possible reason may be possibly due to difference in the level of pesticide use, fodder supply, breed of cow and buffalo chosen for milk collection and health of animal.

The mean β HCH concentration in cow milk was higher (0.179413 mg/ml) in the rainy months compared to summer (0.096336 mg/ml) and winter (0.038392 mg/ml) and data obtained for difference in β HCH concentration in three seasons was found to be highly significant (P value= 0.001; P ≤ 0.01), which means there is a highly significant difference in the level of β HCH in the three seasons. The significant seasonal variation was due to the highly significant β HCH concentration during rainy and summer (P value = 0.000; P ≤ 0.01) during rainy and winter season (P value = 0.000; P ≤0.01).

The mean β HCH concentration in buffalo milk was higher (0.458112 mg/ml) in the winter months compared to summer (0.098010 mg/ml) and rainy (0.267367 mg/ml) and data obtained for difference in β HCH concentration in three seasons was
found highly significant (P value= 0.000; P ≤ 0.01), which means there is a highly significant difference in the level of β HCH in the three seasons. The significant seasonal variation was due to the highly significant the mean difference of β HCH concentration during rainy and winter season was found highly significant (P value = 0.002; P ≤ 0.01) and during summer and winter season (P value = 0.000; P ≤ 0.01). The possible reason for this trend might be that the influences of heat, wind and rain, which are the characteristics of the summer and rainy seasons, are very low during winter seasons so that the dissipation of pesticide residues does not take place very rapidly. This reveals that there is significant effect of seasonal factors on the level of β HCH accumulation in the milk samples in Dhanbad city.

The difference in β HCH concentration in different locations in cow and buffalo milk was found highly significant (P value= 0.000; P ≤ 0.01), which means there is a highly significant level of difference in the level of β HCH both in cow and buffalo milk in the different locations. The possible reason may be possibly due to difference in the level of pesticide use, fodder supply, breed of cow and buffalo chosen for milk collection and health of animal.

The mean γ HCH concentration in cow milk was higher (0.093133 mg/ml) in the rainy months compared to summer (0.008873 mg/ml) and winter (0.035112 mg/ml) and the data obtained for difference in γ HCH concentration in three seasons was found to be highly significant (P value= 0.001; P ≤0.01), which means there is a highly significant difference in the level of γ HCH in the three seasons. The significant seasonal variation was due to the highly significant difference of γ HCH
concentration during rainy and summer season (P value = 0.000; P ≤ 0.01) and significant difference during rainy and winter season (P value = 0.013; P ≤ 0.05).

The mean γ HCH concentration in buffalo milk was higher (0.142550 mg/ml) in the winter months compared to summer (0.020167 mg/ml) and rainy (0.053000 mg/ml) and the data obtained for difference in γ HCH concentration in three seasons was found highly significant (P value= 0.000; P ≤ 0.01), which means there is a highly significant difference in the level of γ HCH in the three seasons. The highly significant seasonal variation was due to the highly significant difference of γ HCH concentration during rainy and winter season (P value = 0.003; P ≤ 0.01). Difference of γ HCH concentration during summer and winter season was also found highly significant (P value = 0.000; P ≤ 0.01). The possible reason for this trend might be that the influences of heat, wind and rain, which are the characteristics of the summer and rainy seasons, are very low during winter seasons so that the dissipation of pesticide residues does not take place very rapidly. This reveals that there is significant effect of seasonal factors on the level of γ HCH accumulation in the milk samples in Dhanbad city.

The difference in γ HCH concentration in different locations in cow and buffalo milk was found highly significant (P value= 0.000; P ≤ 0.01), which means there is a highly significant level of difference in the level of γ HCH both in cow and buffalo milk in the different locations. The possible reason may be possibly due to difference in the level of pesticide use, fodder supply, breed of cow and buffalo chosen for milk collection and health of animal.
The mean $\sum$ HCH concentration in cow milk was higher (0.339717 mg/ml) in the rainy months compared to summer (0.215100 mg/ml) and winter (0.134100 mg/ml) and data obtained for difference in $\sum$ HCH concentration in three seasons was found to be highly significant (P value = 0.001; P ≤ 0.01), which means there is a highly significant difference in the level of $\sum$ HCH in the three seasons. The highly significant seasonal variation was due to the significant difference of $\sum$ HCH concentration during rainy and summer season (P value = 0.020; P ≤ 0.05), highly significant difference of $\sum$ HCH concentration during rainy and winter season (P value = 0.000; P ≤ 0.01) and highly significant difference (P value = 0.000; P ≤ 0.01) during summer and winter season. This reveals that there is significant effect of nature of OCP residue metabolism inside the animal body along with seasonal factors on the level of $\alpha$ HCH accumulation in the milk samples in Dhanbad city.

The mean $\sum$ HCH concentration in buffalo milk was higher (0.458112 mg/ml) in the winter months compared to summer (0.09801 mg/ml) and rainy (0.267367 mg/ml) and the data obtained for difference in $\sum$ HCH concentration in three seasons was found highly significant (P value = 0.000; P ≤ 0.01), which means there is a highly significant difference in the level of $\sum$ HCH in the three seasons. The significant seasonal variation was due to the significant difference of $\sum$ HCH concentration during rainy and summer season (P value = 0.017; P ≤ 0.05), highly significant difference during rainy and winter season significant (P value = 0.007; P ≤ 0.01) and highly significant difference (P value = 0.000; P ≤ 0.01) during summer and winter season. This reveals that there is significant effect of seasonal factors on the level of $\sum$ HCH accumulation in the milk samples in Dhanbad city.
The difference in $\sum$ HCH concentration in different locations in cow and buffalo milk was found highly significant ($P$ value= 0.000; $P \leq 0.01$), which means there is a highly significant level of difference in the level of $\sum$HCH both in cow and buffalo milk in the different locations.

From the above observation, it can be depicted that there is highly significant difference in the level of HCH isomers in three different seasons both in case of cow and buffalo milk. On the other hand, the trend of seasonal variation of HCH isomers was different in cow and buffalo milk. In cow milk, the highest HCH concentration was found in winter months while in buffalo milk, the highest HCH concentration was found in rainy months. This is because the effect of seasonal factors has been more in case of buffalo milk.

The mean $\alpha$ HCH concentration in human milk was higher (0.006068 mg/ml) in the rainy months compared to summer (0.003627 mg/ml) and winter (0.000262 mg/ml) and data obtained for difference in $\alpha$ HCH concentration in three seasons was found highly significant ($P$ value= 0.000; $P \leq 0.01$), which means there is a highly significant difference in the level of $\alpha$ HCH in the three seasons. The highly significant seasonal variation was due to the highly significant difference of $\alpha$ HCH concentration during rainy and winter season ($P$ value = 0.000; $P \leq 0.01$) and significant difference during summer and winter season ($P$ value = 0.019; $P \leq 0.05$).

The mean $\beta$ HCH concentration was higher (0.157005 mg/ml) in the rainy months compared to summer (0.094595 mg/ml) and winter (0.010805 mg/ml) and data obtained for difference in $\beta$ HCH concentration in three seasons was found non significant ($P$ value= 0.106; $P \geq 0.05$), which means there is a non significant
difference in the level of \( \beta \) HCH in the three seasons. The mean \( \gamma \) HCH concentration was higher (0.082330 mg/ml) in the rainy months compared to summer (0.022505 mg/ml) and winter (0.006300 mg/ml) and data obtained for difference in \( \gamma \) HCH concentration in three seasons was found significant (P value= 0.030; \( P \leq 0.05 \)), which means there is a significant difference in the level of \( \gamma \) HCH in the three seasons. The significant seasonal variation was due to significant difference of \( \gamma \) HCH concentration during rainy and summer season (P value = 0.046; \( P \leq 0.05 \)) and during rainy and winter season (P value = 0.012; \( P \leq 0.05 \)). The mean \( \sum \) HCH concentration was higher (0.245403 mg/ml) in the rainy months compared to summer (0.120727 mg/ml) and winter (0.017367 mg/ml and the data obtained for difference in \( \sum \) HCH concentration in three seasons was found to be significant (P value= 0.014; \( P \leq 0.05 \)), which means there is a significant difference in the level of \( \sum \) HCH in the three seasons. The significant seasonal variation was due to the highly significant difference of \( \sum \) HCH concentration during rainy and winter season (P value = 0.004; \( P \leq 0.01 \)).

From the above observation, it can be depicted that there is significant difference in the level of HCH isomers in three different seasons in case of human milk. This reveals that there is significant effect of nature of OCP residue metabolism inside the animal body along with seasonal factors on the level of HCH accumulation in the milk samples in Dhanbad city.

The difference in \( \alpha \) HCH concentration in different locations in human milk was found to be non significant (P value= 0.243; \( P \geq 0.05 \)), which means there is a no significant difference in the level of \( \alpha \) HCH in the different locations. The difference
in $\beta$ HCH concentration in different locations was found highly significant ($P$ value= 0.000; $P \leq 0.01$), which means there is a highly significant level of difference in the level of $\beta$ HCH in the different locations. The difference in $\gamma$ HCH concentration in different locations was found highly significant ($P$ value= 0.000; $P \leq 0.01$), which means there is a highly significant level of difference in the level of $\gamma$ HCH in the different locations. The difference in $\sum$ HCH concentration in different locations was found highly significant ($P$ value= 0.002; $P \leq 0.01$), which means there is a highly significant level of difference in the level of HCH in the different locations. This may be due to the difference in food habit and health of the mothers from whom milk had been collected.

The mean $p,p'$-DDE concentration in cow milk was higher (0.148917 mg/ml) in the winter months compared to summer (0.083073 mg/ml) and rainy (0.021450 mg/ml) and data obtained for difference in $p,p'$-DDE concentration in three seasons was found to be highly significant ($P$ value= 0.001; $P \leq 0.01$), which means there is a highly significant difference in the level of $p,p'$-DDE in the three seasons. The highly significant seasonal variation was due to the highly significant ($P$ value = 0.000; $P \leq 0.01$) difference of $p,p'$-DDE concentration during rainy and winter season. This might possibly be due to the influences of heat, wind and rain, which are the characteristics of the summer and rainy seasons, are very low during winter seasons so that the dissipation of pesticide residues does not take place very rapidly. This reveals that there is significant effect of seasonal factors on the level of $p,p'$-DDE accumulation in the milk samples in Dhanbad city.
The mean p,p’-DDE concentration in buffalo milk was higher (0.154317 mg/ml) in the winter months compared to summer (0.065948 mg/ml) and rainy (0.018863 mg/ml) and data obtained for difference in p,p’-DDE concentration in three seasons was found highly significant (P value= 0.000; P ≤ 0.01), which means there is a highly significant difference in the level of p,p’-DDE in the three seasons. The highly significant seasonal variation was due to the highly significant difference of p,p’-DDE concentration during rainy and summer season (P value = 0.000; P ≤ 0.01) and during summer and winter season (P value = 0.009; P ≤ 0.01). The possible reason for this trend may be due lesser precipitation of OCP residues during the winter months. This reveals that there is significant effect of seasonal factors on the level of p,p’-DDE accumulation in the milk samples in Dhanbad city.

The difference in p,p’-DDE concentration in different locations in cow and buffalo milk was found highly significant (P value= 0.000; P ≤ 0.01), which means there is a highly significant difference for p,p’-DDE both in cow and buffalo milk in the different locations. The possible reason may be possibly due to difference in the level of pesticide use, fodder supply, breed of cow and buffalo chosen for milk collection and health of animal.

The mean p,p’-DDD concentration in cow milk was higher (0.027550 mg/ml) in the winter months compared to summer (0.008518 mg/ml) and rainy (0.001750 mg/ml) and data obtained for difference in p,p’-DDD concentration in three seasons was found to be highly significant (P value= 0.001; P ≤ 0.01), which means there is a highly significant difference in the level of p,p’-DDD in the three seasons. The highly significant seasonal variation was due to the highly significant difference of
p,p’-DDD concentration during rainy and winter season (P value = 0.000; P ≤ 0.01) and summer and winter season (P value = 0.000; P ≤ 0.01). The possible reason for this trend might be similar to that stated in case of p,p’-DDE. This reveals that there is significant effect of seasonal factors on the level of p,p’-DDD accumulation in the milk samples in Dhanbad city.

The mean p,p’-DDD concentration in buffalo milk was higher (0.045017 mg/ml) in the winter months compared to summer (0.006667 mg/ml) and rainy (0.017175 mg/ml) and data obtained for difference in p,p’-DDD concentration in three seasons was found significant (P value= 0.011; P ≤ 0.05), which means there is a significant difference in the level of p,p’-DDD in the three seasons. The significant seasonal variation was due to the significant difference of p,p’-DDD concentration during rainy and winter season (P value = 0.035; P ≤ 0.05) and during summer and winter season (P value = 0.004; P ≤ 0.01). The possible reasons for this trend might be similar to that stated in case of cow milk. This reveals that there is significant effect of seasonal factors on the level of p,p’-DDD accumulation in the milk samples in Dhanbad city.

The difference in p,p’-DDD concentration in different locations in cow and buffalo milk was found non significant (P value= 0.090; P ≥ 0.05) and (P value= 0.098; P ≥0.05), respectively, which means there is non significant level of difference in the level of p,p’-DDD in the different locations. The possible reason may be possibly due to difference in the level of pesticide use, fodder supply, breed of cow and buffalo chosen for milk collection and health of animal.
The mean o,p’-DDT concentration in cow milk was higher (0.003487 mg/ml) in the winter months compared to summer (0.001827 mg/ml) and rainy (0.001140 mg/ml) and data obtained for difference in o,p’-DDT concentration in three seasons was found significant (P value= 0.047; P ≤ 0.05), which means there is a significant difference in the level of o,p’-DDT in the three seasons. The significant seasonal variation was due to the significant difference of o,p’-DDT concentration during rainy and winter season (P value = 0.016; P ≤ 0.05). This reveals that there is significant effect of seasonal factors on the level of o,p’-DDT accumulation in the milk samples in Dhanbad city.

The mean o,p’-DDT concentration in buffalo milk was higher (0.004423 mg/ml) in the winter months compared to summer (0.000305 mg/ml) and rainy (0.001237 mg/ml) and the data obtained for difference in o,p’-DDT concentration in three seasons was found highly significant (P value= 0.002; P ≤ 0.01), which means there is a highly significant difference in the level of o,p’-DDT in the three seasons. The highly significant seasonal variation was due to the highly significant difference of o,p’-DDT concentration during rainy and winter season (P value = 0.008; P ≤ 0.01) and during summer and winter season (P value = 0.001; P ≤ 0.01). The possible reason for this might be attributed to the nature of p,p’-DDD metabolism inside the body. This reveals that there is significant effect of seasonal factors on the level of o,p’-DDT accumulation in the milk samples in Dhanbad city.

The data obtained for difference in o,p’-DDT concentration in different locations in both cow and buffalo milk was found highly significant (P value= 0.003; P ≤ 0.01) and (P value= 0.000; P ≤ 0.01), respectively, which means there is a highly
significant level of difference in the level of o,p’-DDT in the different locations. The possible reason may be possibly due to difference in the level of pesticide use, fodder supply, breed of cow and buffalo chosen for milk collection and health of animal.

The mean p,p’-DDT concentration in cow milk was higher (0.031883 mg/ml) in the winter months compared to summer (0.020183 mg/ml) and rainy (0.019373 mg/ml) and data obtained for difference in p,p’-DDT concentration in three seasons was found to be non significant (P value= 0.278; P ≥0.05), which means there is non significant difference in the level of p,p’-DDT in the three seasons. This may be attributed to nature of OCP residue metabolism inside the animal body. This reveals that there is non significant effect of seasonal factors on the level of α HCH accumulation in the milk samples in Dhanbad city.

The mean p,p’-DDT concentration in buffalo milk was higher (0.043467 mg/ml) in the winter months compared to summer (0.012250 mg/ml) and rainy (0.020917 mg/ml) and the data obtained for difference in p,p’-DDT concentration in three seasons was found significant (P value= 0.017; P ≤0.05), which means there is a significant difference in the level of p,p’-DDT in the three seasons. The significant seasonal variation was due to the significant difference of p,p’-DDT concentration during rainy and winter season (P value = 0.044; P ≤ 0.05) and highly significant difference during summer and winter season (P value = 0.006; P ≤0.01). The possible reason for this trend might be that the influences of heat, wind and rain, which are the characteristics of the summer and rainy seasons, are very low during winter seasons so that the dissipation of pesticide residues does not take place very rapidly. This
reveals that there is significant effect of seasonal factors on the level of p,p’-DDT accumulation in the milk samples in Dhanbad city.

The difference in p,p’-DDT concentration in different locations in both cow and buffalo milk was found highly significant (P value= 0.000; P ≤ 0.01) and (P value= 0.008; P ≤0.01), respectively, which means there is a highly significant level of difference in the level of p,p’-DDT in the different locations. The possible reason may be possibly due to difference in the level of pesticide use, fodder supply, breed of cow and buffalo chosen for milk collection and health of animal.

The mean ∑ DDT concentration in cow milk was higher (0.211837 mg/ml) in the winter months compared to summer (0.113602 mg/ml) and rainy (0.043713 mg/ml) and data obtained for difference in ∑ DDT concentration in three seasons was found highly significant (P value= 0.001; P ≤ 0.01), which means there is a highly significant difference in the level of ∑ DDT in the three seasons. The highly significant seasonal variation was due to the significant difference of ∑ DDT concentration during rainy and winter season (P value = 0.000; P ≤0.01) and during summer and winter season (P value = 0.010; P ≤0.01). The suitable reasons for this trend have been mentioned earlier. This reveals that there is significant effect of seasonal factors on the level of ∑ DDT accumulation in the milk samples in Dhanbad city.

The mean ∑ DDT concentration in buffalo milk was higher (0.247223 mg/ml) in the winter months compared to summer (0.085170 mg/ml) and rainy (0.058192 mg/ml) and the data obtained for difference in ∑ DDT concentration in three seasons was found significant (P value= 0.013; P ≤0.05), which means there is
a significant difference in the level of $\sum$ DDT in the three seasons. The significant seasonal variation was due to the significant difference of $\sum$ DDT concentration during rainy and summer season ($P$ value = 0.007; $P \leq 0.01$) and during rainy and winter season ($P$ value = 0.017; $P \leq 0.05$). This reveals that there is significant effect of seasonal factors on the level of $\sum$ DDT accumulation in the milk samples in Dhanbad city.

The difference in $\sum$ DDT concentration in different locations in both cow and buffalo milk was found highly significant ($P$ value= 0.000; $P \leq 0.01$), which means there is a highly significant level of difference in the level of $\sum$ DDT in the different locations.

From the above observation, it can be depicted that there is highly significant difference in the level of DDT and its isomers in three different seasons both in case of cow and buffalo milk. The trend of seasonal variation of DDT isomers was similar in both cow and buffalo milk, i.e. the concentration of DDT and its isomers was maximum in the winter season.

The mean p,p’-DDE concentration in human milk was higher (1.085300 mg/ml) in the winter months compared to summer (0.993955 mg/ml) and rainy (0.897500 mg/ml) and data obtained for difference in p,p’-DDE concentration in three seasons was found non significant ($P$ value= 0.950; $P \geq 0.05$), which means there is no significant difference in the level of p,p’-DDE in the three seasons. The mean p,p’-DDD concentration was higher (0.032319 mg/ml) in the winter months compared to summer (0.025696 mg/ml) and rainy (0.017720 mg/ml) and data obtained for difference in p,p’-DDD concentration in three seasons was found non
significant (P value= 0.594; P ≥0.05), which means there is no significant difference in the level of p,p’-DDD in the three seasons. The mean o,p’-DDT concentration was higher (0.000580 mg/ml) in the winter months compared to summer (0.000490 mg/ml) and rainy (0.000335 mg/ml) and data obtained for difference in o,p’-DDT concentration in three seasons was found non significant (P value= 0.831; P ≥0.05), which means there is no significant difference in the level of o,p’-DDT in the three seasons. The mean p,p’-DDT concentration was higher (1.168650 mg/ml) in the winter months compared to summer (1.082400 mg/ml) and rainy (0.822800 mg/ml) and winter (0.017367 mg/ml) and the data obtained for difference in p,p’-DDT concentration in three seasons was found non significant (P value= 0.856; P ≥0.05), which means there is no significant difference in the level of p,p’-DDT in the three seasons. The mean \( \sum \) DDT concentration was higher (2.286849 mg/ml) in the winter months compared to summer (2.102541 mg/ml) and rainy (1.738355 mg/ml) and data obtained for difference in \( \sum \) DDT concentration in three seasons was found non significant (P value= 0.904; P ≥0.05), which means there is no significant difference in the level of \( \sum \) DDT in the three seasons.

From the above observation, it can be depicted that there is non significant difference in the level of DDT and its isomers in three different seasons in case of human milk. This reveals that there is non significant effect of seasonal factors on the level of DDT accumulation in the milk samples in Dhanbad city.

The difference in p,p’-DDE concentration in different locations in human milk was found non significant (P value= 0.409; P ≥0.05), which means there is a non significant difference in the level of p,p’-DDE in the different locations. The
difference in p,p’-DDD concentration in different locations was found non significant (P value= 0.168; P ≥ 0.05), which means there is a non significant difference in the level of p,p’-DDD in the different locations. The difference in o,p’-DDT concentration in different locations was found non significant (P value= 0.463; P ≥ 0.05), which means there is a non significant difference in the level of o,p’-DDT in the different locations. The difference in p,p’-DDT concentration in different locations was found to be non significant (P value= 0.380; P ≥ 0.05), which means there is a non significant difference in the level of p,p’-DDT in the different locations. The difference in ∑ DDT concentration in different locations was found non significant (P value= 0.387; P ≥ 0.05), which means there is a non significant difference in the level of ∑ DDT in the different locations.

The mean α-Endosulfan concentration in cow milk was higher (0.002600 mg/ml) in the winter months compared to summer (0.001983 mg/ml) and rainy (0.001250 mg/ml) and data obtained for difference in α-Endosulfan concentration in three seasons was found non significant (P value= 0.457; P ≥ 0.05), which means there is non significant difference in the level of α-Endosulfan in the three seasons. This may be attributed to nature of OCP residue metabolism inside the animal body. This reveals that there is non significant effect of seasonal factors on the level of p,p’-DDE accumulation in the milk samples in Dhanbad city.

The mean α-Endosulfan concentration in buffalo milk was higher (0.005017 mg/ml) in the winter months compared to summer (0.003667 mg/ml) and rainy (0.002700 mg/ml) and data obtained for difference in α-Endosulfan concentration in three seasons was found non significant (P value= 0.529; P ≥ 0.05), which means
there is no significant difference in the level of $\alpha$-Endosulfan in the three seasons. This may be due to the nature of OCP residue metabolism inside the animal body. This reveals that there is no significant effect of seasonal factors on the level of p,p'-DDE accumulation in the milk samples in Dhanbad city.

The difference in $\alpha$-Endosulfan concentration in different locations in both cow and buffalo milk was found significant ($P$ value= 0.016; $P \leq 0.05$) and ($P$ value= 0.021; $P \leq 0.05$), which means there is a significant level of difference in the level of $\alpha$-Endosulfan in the different locations. The possible reason may be possibly due to difference in the level of pesticide use, fodder supply, breed of cow and buffalo chosen for milk collection and health of animal.

The mean $\beta$-Endosulfan concentration in cow milk was higher (0.002893 mg/ml) in the winter months compared to summer (0.002473 mg/ml) and rainy (0.001535 mg/ml) and data obtained for difference in $\beta$-Endosulfan concentration in three seasons was found non significant ($P$ value= 0.610; $P \geq 0.05$), which means there is no significant difference in the level of $\beta$-Endosulfan in the three seasons. The possible reason for this trend may be the nature of OCP residue metabolism inside the animal body. This reveals that there is no significant effect of seasonal factors on the level of $\beta$-Endosulfan accumulation in the milk samples in Dhanbad city.

The mean $\beta$-Endosulfan concentration in buffalo milk was higher (0.004321 mg/ml) in the winter months compared to summer (0.003031 mg/ml) and rainy (0.002268 mg/ml) and data obtained for difference in $\beta$-Endosulfan concentration in three seasons was found non significant ($P$ value= 0.421; $P \geq 0.05$), which means
there is non significant difference in the level of $\beta$-Endosulfan in the three seasons. This may be attributed to nature of OCP residue metabolism inside the animal body. This reveals that there is non significant effect of seasonal factors on the level of $\beta$-Endosulfan accumulation in the milk samples in Dhanbad city.

The difference in $\beta$-Endosulfan concentration in different locations in cow and buffalo milk was found significant (P value= 0.016; P $\leq$ 0.05) and highly significant (P value= 0.000; P $\leq$0.01), respectively, which means there is a significant level of difference in the level of $\beta$-Endosulfan in the different locations. The possible reason may be possibly due to difference in the level of pesticide use, fodder supply, breed of cow and buffalo chosen for milk collection and health of animal.

The mean Endosulfan Sulfate concentration in cow milk was higher (0.000465 mg/ml) in the winter months compared to summer (0.000308 mg/ml) and rainy (0.000147 mg/ml) and data obtained for difference in Endosulfan Sulfate concentration in three seasons was found non significant (P value= 0. 210; P $\geq$0.05), which means there is no significant difference in the level of Endosulfan Sulfate in the three seasons. This reveals that there is non significant effect of seasonal factors on the level of Endosulfan Sulfate accumulation in the milk samples in Dhanbad city.

The mean Endosulfan Sulfate concentration in buffalo milk was higher (0.000735 mg/ml) in the winter months compared to summer (0.000413 mg/ml) and rainy (0.000365 mg/ml) and data obtained for difference in Endosulfan Sulfate concentration in three seasons was found non significant (P value= 0.220; P $\geq$0.05), which means there is non significant difference in the level of Endosulfan Sulfate in the three seasons. The possible reason may be due to nature of OCP residue
metabolism inside the animal body. This reveals that there is non significant effect of seasonal factors on the level of Endosulfan Sulfate accumulation in the milk samples in Dhanbad city.

The data obtained for difference in Endosulfan Sulfate concentration in different locations in cow milk was found non significant (P value= 0.083; P ≥0.05), which means there is non significant difference in the level of Endosulfan Sulfate in the different locations while the data obtained for difference in Endosulfan Sulfate concentration in different locations was found significant (P value= 0.036; P ≤0.05), which means there is a significant level of difference in the level of Endosulfan Sulfate in the different locations.

The mean $\sum$ Endosulfan concentration in cow milk was higher (0.005958 mg/ml) in the winter months compared to summer (0.004765 mg/ml) and rainy (0.002931 mg/ml) and data obtained for difference in $\sum$ Endosulfan concentration in three seasons was found non significant (P value= 0.456; P ≥ 0.05), which means there is non significant difference in the level of $\sum$ Endosulfan in the three seasons. This reveals that there is non significant effect of seasonal factors on the level of $\sum$ Endosulfan accumulation in the milk samples in Dhanbad city.

The mean $\sum$ Endosulfan concentration in buffalo milk was higher (0.010073 mg/ml) in the winter months compared to summer (0.007111 mg/ml) and rainy (0.005333 mg/ml) and data obtained for difference in $\sum$ Endosulfan concentration in three seasons was found non significant (P value= 0.362; P ≥ 0.05), which means there is non significant difference in the level of $\sum$ Endosulfan in the three seasons.
This reveals that there is non significant effect of seasonal factors on the level of p,p’-DDT accumulation in the milk samples in Dhanbad city.

The difference in $\sum$ Endosulfan concentration in different locations in cow milk was found significant (P value= 0.017; $P \leq 0.05$) and highly significant (P value= 0.001; $P \leq 0.01$) in buffalo milk, which means there is a significant level of difference in the level of $\sum$ Endosulfan in the different locations.

From the above observation, it can be depicted that there is non significant difference in the level of endosulfan isomers in three different seasons both in case of cow and buffalo milk. The trend of seasonal variation of DDT isomers was similar in both cow and buffalo milk, i.e. the concentration of endosulfan isomers was maximum in the winter season.

$\alpha$-Endosulfan and $\beta$-Endosulfan residue were not detected in case of human milk at all places of study throughout the year.

The mean Endosulfan Sulfate concentration in human milk was higher (0.000080 mg/ml) in the winter months compared to summer (0.000075 mg/ml) and rainy (0.000060 mg/ml) and data obtained for difference in Endosulfan Sulfate concentration in three seasons was found non significant (P value= 0.925; $P \geq 0.05$), which means there is non significant difference in the level of Endosulfan Sulfate in the three seasons. The mean $\sum$ Endosulfan concentration was higher (0.000080 mg/ml) in the winter months compared to summer (0.000075 mg/ml) and rainy (0.000060 mg/ml) and data obtained for difference in $\sum$ Endosulfan concentration in three seasons was found non significant (P value= 0.925; $P \geq 0.05$), which means there is non significant difference in the level of $\sum$ Endosulfan in the three seasons.
From the above observation, it can be depicted that there is non significant difference in the level of endosulfan isomers in three different seasons in case of human milk. This reveals that there is non significant effect of seasonal factors on the level of endosulfan isomers accumulation in the milk samples in Dhanbad city.

The difference in Endosulfan Sulfate and $\sum$ Endosulfan concentration in different locations in human milk was found non significant (P value= 0.412; P $\geq$ 0.05), which means there is a non significant difference in the level of Endosulfan Sulfate in the different locations. The suitable reasons for this trend have been mentioned earlier. This reveals that there is non significant effect of seasonal factors on the level of endosulfan isomers accumulation in the milk samples in Dhanbad city.

**Correlation of OCP residues for all seasons**

From the correlation analysis of OCP residues for all seasons in cow, buffalo and human milk, both positive and negative correlation was found among the various OCP residues. It was also found that all the isomers of OCP residues like for DDT, HCH and Endosulfan, showed a positive correlation. The possible reason for this might be due to the fact that the isomers of the pesticides are produced as a result of metabolic conversion of the respective same technical grade pesticide.
Comparison of pesticide residue level with Maximum Residue Limit (MRL) with reference to possible health hazards in human/infants

The mean concentration of aldrin (mg/ml) detected in the cow milk samples was 0.101896 mg/ml that does not exceed the MRL of aldrin (0.15 mg/ml) as recommended by WHO/FAO/FSSAI. It was also found that out of total 540 samples analyzed, in 72 samples the aldrin level exceeded the MRL for aldrin as per the recommendation of WHO/FAO/FSSAI. The locations affected with high aldrin contamination were C (Bartand), D (Jharudih), F (Hirapur). The mean concentration of aldrin (mg/ml) detected in the buffalo milk samples is 0.132343 mg/ml that does not exceed the MRL of aldrin (0.15 mg/ml) as per the recommendation of WHO/FAO/FSSAI. It was also found that out of total 540 samples analyzed, in 126 samples the aldrin level exceeded the MRL for aldrin as recommended by WHO/FAO/FSSAI. The locations affected with high aldrin contamination were C (Bartand), D (Jharudih), F (Hirapur). It was found that out of total 60 samples analyzed, none of the samples showed the presence of detectable amount of aldrin concentration. The possible reasons may be limited recent aldrin exposure and its rapid breakdown hence elimination from the body.

The mean concentration of HCH (mg/ml) detected in the cow milk samples is 0.229639 mg/ml that exceeded the MRL of HCH (0.10 mg/ml) as per the recommendation of WHO/FAO/FSSAI. It was also found that out of total 540 samples analyzed, in 270 samples the HCH level exceeded the MRL for HCH as per the recommendation of WHO/FAO/FSSAI. The locations affected with high HCH contamination were B (Bhuipore), E (Rangatand), F (Hirapur), G (Bank More), H
(Wasseypur), I (Matkuriya), J (Dhaunsar). The mean concentration of HCH (mg/ml) detected in the buffalo milk samples 0.274496 mg/ml that exceeded the MRL of HCH (0.10 mg/ml) as recommended by WHO/FAO/FSSAI. It was also found that out of total 540 samples analyzed, in 252 samples the HCH level exceeded the MRL for HCH as recommended by WHO/FAO/FSSAI. The locations affected with high HCH contamination were B (Bhuipore), E (Rangatand), G (Bank More), H (Wasseypur), J (Dhaunsar). The mean concentration of HCH (mg/ml) detected in the human milk samples 0.127832 mg/ml that exceeded the MRL of HCH (0.10 mg/ml) as recommended by WHO/FAO/FSSAI. It was also found that out of total 60 samples analyzed, in 24 samples the HCH level exceeded the MRL for HCH as recommended by WHO/FAO/FSSAI. The hospitals and nursing home from where high HCH contamination were detected in human milk samples were H2 (Park Clinic Nursing Home, Hirapur area), H3 (Dwarka Das Jalan Memorial Hospital, Bartand area), H4 (R C Hazra Memorial Hospital, Bank More area). The possible reasons may be recent exposure as well as the high persistence nature of HCH and its isomers as compared to other OCPs.

The mean concentration of DDT (mg/ml) detected in the cow milk samples is 0.123051 mg/ml that does not exceed the MRL of DDT (1.25 mg/ml) as per the recommendation of WHO/FAO/FSSAI. It was also found that out of total 540 samples analyzed, in none of the samples the DDT level exceeded the MRL for DDT as per the recommendation of WHO/FAO/FSSAI. None of the locations in Dhanbad city were affected with very high DDT contamination. The mean concentration of DDT (mg/ml) detected in the buffalo milk samples 0.130195 mg/ml that does not exceed the MRL of DDT (1.25 mg/ml) as per the recommendation of
WHO/FAO/FSSAI. It was also found that out of total 540 samples analyzed, in none of the samples the DDT level exceeded the MRL for DDT as per the recommendation of WHO/FAO/FSSAI. None of the locations in Dhanbad city were affected with very high DDT contamination. The mean concentration of DDT (mg/ml) detected in the human milk samples 2.042582 mg/ml that exceeded the MRL of DDT (1.25 mg/ml) as recommended by WHO/FAO/FSSAI. It was also found that out of total 60 samples analyzed, in 15 samples the DDT level exceeded the MRL for DDT recommended by WHO/FAO/FSSAI. The hospitals and nursing home from where high DDT contamination were detected in human milk samples were H2 (Park Clinic Nursing Home, Hirapur area), H3 (Dwarka Das Jalan Memorial Hospital, Bartand area), H4 (R C Hazra Memorial Hospital, Bank More area). The possibility may be for improper use of technical grade DDT used in recent times and the higher biomagnification of DDT and its isomers with increasing trophic level.

The mean concentration of Endosulfan (mg/ml) detected in the cow milk samples is 0.004552 mg/ml that does not exceed the MRL of Endosulfan (0.01 mg/ml) as per the recommendation of WHO/FAO/FSSAI. It was also found that out of total 540 samples analyzed, in 45 samples the Endosulfan level exceeded the MRL for Endosulfan as per the recommendation of WHO/FAO/FSSAI. The locations affected with high Endosulfan contamination were B (Bhuipore), D (Jharudih). The mean concentration of Endosulfan (mg/ml) detected in the buffalo milk samples is 0.007506 mg/ml that does not exceed the MRL of Endosulfan (0.01 mg/ml) as per the recommendation of WHO/FAO/FSSAI. It was also found that out of total 540 samples analyzed, in 108 samples the Endosulfan level exceeded the MRL for
Endosulfan as recommended by WHO/FAO/FSSAI. The locations affected with high Endosulfan contamination were B (Bhuipore), D (Jharudih), E (Rangatand). The mean concentration of Endosulfan (mg/ml) detected in the human milk samples 0.000072 mg/ml that exceeded the MRL of Endosulfan (0.01 mg/ml) as recommended by WHO/FAO/FSSAI. It was also found that out of total 60 samples analyzed, in none of the samples the Endosulfan level exceeded the MRL for Endosulfan recommended by WHO/FAO/FSSAI.

From the above observation, it can be depicted that bovine and human milk in Dhanbad city were contaminated with residues of highly persistent OCPs like aldrin, HCH, DDT and endosulfan. This indicates an alarming risk to the health of humans/children population of Dhanbad city due to pesticide exposure. The acute health effects of OCP residues detected may be irritation of the skin, nausea, dizziness, diarrhea and severe reactions to people having asthma whereas the chronic health effects may include cancer and other tumors, brain and nervous system damage, birth defects, infertility and other reproductive problems, damage to the liver, kidneys, lungs and other body organs.
Table S.1 Seasonal variation of OCP residues

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Cow Variation</th>
<th>Buffalo Variation</th>
<th>Human Variation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>W&gt;S&gt;R Sig</td>
<td>W&gt;S&gt;R Sig**</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>HCH</td>
<td>R&gt;S&gt;W Sig**</td>
<td>W&gt;R&gt;S Sig**</td>
<td>R&gt;S&gt;W Sig</td>
<td></td>
</tr>
<tr>
<td>DDT</td>
<td>W&gt;S&gt;R Sig**</td>
<td>W&gt;S&gt;R Sig</td>
<td>W&gt;S&gt;R Non Sig</td>
<td></td>
</tr>
<tr>
<td>Endosulfan</td>
<td>W&gt;S&gt;R Non Sig</td>
<td>W&gt;S&gt;R Non Sig</td>
<td>W&gt;S&gt;R Non Sig</td>
<td></td>
</tr>
</tbody>
</table>

Table S.2 Comparison of OCP residues in cow milk with recommended MRL (WHO/FAO/FSSAI)

<table>
<thead>
<tr>
<th>Pesticide (mg/l)</th>
<th>Cow</th>
<th>WHO/FAO</th>
<th>FSSAI</th>
<th>Above MRL (Yes/No)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>0.101896</td>
<td>0.15</td>
<td>0.15</td>
<td>No</td>
</tr>
<tr>
<td>HCH</td>
<td>0.229639</td>
<td>0.10</td>
<td>0.10</td>
<td>Yes</td>
</tr>
<tr>
<td>DDT</td>
<td>0.123051</td>
<td>1.25</td>
<td>1.25</td>
<td>No</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>0.004552</td>
<td>0.01</td>
<td>0.01</td>
<td>No</td>
</tr>
</tbody>
</table>

Table S.3 Comparison of OCP residues in buffalo milk with recommended MRL (WHO/FAO/FSSAI)

<table>
<thead>
<tr>
<th>Pesticide (mg/l)</th>
<th>Buffalo</th>
<th>WHO/FAO</th>
<th>FSSAI</th>
<th>Above MRL (Yes/No)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>0.132343</td>
<td>0.15</td>
<td>0.15</td>
<td>No</td>
</tr>
<tr>
<td>HCH</td>
<td>0.274496</td>
<td>0.10</td>
<td>0.10</td>
<td>Yes</td>
</tr>
<tr>
<td>DDT</td>
<td>0.130195</td>
<td>1.25</td>
<td>1.25</td>
<td>No</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>0.007506</td>
<td>0.01</td>
<td>0.01</td>
<td>No</td>
</tr>
</tbody>
</table>

Table S.4 Comparison of OCP residues in human milk with recommended MRL (WHO/FAO/FSSAI)

<table>
<thead>
<tr>
<th>Pesticide (mg/l)</th>
<th>Human</th>
<th>WHO/FAO</th>
<th>FSSAI</th>
<th>Above MRL (Yes/No)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCH</td>
<td>0.127832</td>
<td>0.10</td>
<td>0.10</td>
<td>Yes</td>
</tr>
<tr>
<td>DDT</td>
<td>2.042582</td>
<td>1.25</td>
<td>1.25</td>
<td>Yes</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>0.000072</td>
<td>0.01</td>
<td>0.01</td>
<td>No</td>
</tr>
</tbody>
</table>
CONCLUSION

- The bovine (cow and buffalo) milk samples collected from different locations (area) of Dhanbad city was observed and found contaminated with OCP residues of Aldrin, α HCH, β HCH, γ HCH, p,p’-DDE, p,p’-DDD, o,p’-DDT, p,p’-DDT, α Endosulfan, β Endosulfan and Endosulfan Sulfate.

- The human milk sample was contaminated with OCP residues of α HCH, β HCH, γ HCH, p,p’-DDE, p,p’-DDD, o,p’-DDT, p,p’-DDT and Endosulfan Sulfate.

- The OCP residues enter the animal’s body through taking contaminated feed, drinking contaminated water, breathing contaminated air or by coming into contact with contaminated soil near hazardous waste sites. Once these OCPs enter the animal body, due to their lipophilic nature, they are not easily removed or degraded and gets accumulated in the fatty tissues and further are excreted with the milk, making it contaminated.

- The presence of OCP residues in bovine and human milk indicates OCP exposure and hence residue contamination in and around Dhanbad city.

- In bovine milk, the highest concentration of OCP residue was that of HCH followed by DDT. This may be due to high use of these pesticides both in past and in recent time, high nature of persistence and longer half life as compared to other OCPs.

- In human milk, the highest concentration of OCP residue was that of DDT. This may be because of recent exposure to DDT and biomagnification inside human body.
The level of persistence of HCH and DDT and their conversion into respective metabolites was high throughout the city due to greater stability of the isomers.

The locations (areas) in the city showing high OCP contamination are Bhuipore (B), Bartand (C), Jharudih (D) and Dhaunsar (J).

In case of both bovine and human milk, regular trend of seasonal variation of OCP residue was seen i.e. the concentration of OCP residue was maximum during the winter season followed by summer and rainy season because the influences of heat, wind and rain, which are the characteristics of summer and rainy seasons, are very low during winter season, so that dissipation of OCPs does not take place rapidly from the environment. Also as intake of fodder in cattles is more during the winter season, this increases the intake of OCPs.

In bovine milk, there is significant difference in the level of aldrin, HCH and DDT in different seasons whereas there is non significant difference in case of endosulfan.

In human milk, there is significant difference in the level of only HCH in different seasons whereas there is non significant difference in case of DDT and endosulfan.

All the isomers of OCP residues like for DDT, HCH and Endosulfan, showed a positive correlation amongst respective OCP isomers because the isomers of the pesticides are produced as a result of metabolic conversion of the respective same technical grade pesticide.

In bovine milk, only mean concentration of HCH (mg/ml) exceeded the MRL of HCH (0.10 mg/ml) as recommended by WHO/FAO/FSSAI whereas in
human milk, both mean concentration of HCH and DDT (mg/ml) exceeded the MRL of HCH (0.10 mg/ml) and DDT (1.25 mg/ml) as recommended by WHO/FAO/FSSAI.

- The population of Dhanbad city is at a high risk of both HCH and DDT exposure. The people are likely to suffer from the various acute as well as chronic effects of HCH and DDT exposure such as irritation of the nose, throat, and skin causing burning, stinging and itching as well as rashes and blisters, nausea, dizziness, diarrhea, severe reactions to people having asthma and cancer and other tumors, brain and nervous system damage, birth defects, infertility and other reproductive problems, damage to the liver, kidneys, lungs and other body organs.