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Determination of organophosphorus pesticide residues in milk samples along Musi river belt, Hyderabad, India

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Abstract
The present study was conducted to find the organophosphorus pesticide (OPP) residues in milk samples along Musi river belt, India. Milk samples collected from the six zones of Musi river belt, Hyderabad India were analyzed by gas chromatography with electron capture detector and pulsated flame photometric detector for the presence of OPP residues. The gas chromatographic analysis of milk samples of Zone IV of Musi river showed the residues of Quinolphos at concentration of Quinolphos 0.06 ± 0.015 (0.04-0.08) ppm. The residues of Dichlorovas, Phorate, Methyl parathion, Fenitrotion, Profenphos, Ethion, Phosalone, Lambda Cyhalotrin were below the detection limit in the remaining milk samples collected from Musi river belt in the present study. The results indicate that the pesticide residues in milk samples were well below the maximum residue level (MRL) values, whereas Quinolphos in milk was slightly above the MRL values specified by European Union (EU).

Keywords: gas chromatography, milk, Musi river, pesticide residues.

1. Introduction
Milk has been studied as an indicator of the bio-concentration of environmentally persistent organic micro-pollutants such as pesticides [1]. The substances intended for preventing, destroying, repelling any 'pest' are known as pesticides. Several hundred pesticides of different chemical nature are currently used for agricultural purposes all over the world. Because of their widespread use, they are detected in various environmental matrices, such as soil, water and air. Pesticides are divided into many classes, of which the most important are organochlorine and organophosphorous compounds. Organophosphorus (OP) pesticides are esters, amides, or thiol derivatives of phosphoric or phosphonic acid. OP pesticides are easily hydrolyzed and therefore do not persist in the environment. However, their toxicity (high or moderate) and the possibility of their accumulation especially fat-soluble OPs in animal tissues, milk, and eggs pose risks for human health [2]. The presence of pesticide residues in milk is a public health concern because milk and dairy products are widely consumed by infants, children, and adults. Pesticide residues in milk originate from contaminated feed, grass or corn silage, and direct application of pesticides on dairy cattle. Because humans are the last link in the food chain, they consume the highest levels of these compounds. Food products particularly dairy, meat, and fish are the primary immediate sources of OC and OP pesticide intake in the general population [3, 4] and these pesticides elicit a wide range of toxic and biochemical effects in both laboratory animals and wildlife [5, 6].
Most of the time, the presence of pesticide residues in animal feeds is the main source of pesticide contamination of dairy products, but other factors may include environmental contamination, application of pesticides on farm animals for ectoparasite removal and accidental spills [7], Pesticides monitory studies are useful to know the level and the main sources of milk and dairy product contamination, and the more research on this subject, the more reliable are the data generated. Furthermore, these data can be used for prevention and control of chemical contaminants in milk and dairy products [8].The dilemma of cost/efficacy, versus ecological impacts, including long range transport, and access to modern pesticides formulations at low cost remains a contentious global issue [9]. Due to the lipophilic nature of these pesticides, milk and other fat-rich substances are the key items for their accumulation. These toxicants get into the human body through the food chain and cause serious health problems [10].
Musi River is located on the Deccan platue in the state of Telangana, India. However, now the

Water is highly polluted as 600 million liters per day of untreated sewage water is discharged into Musi River, additionally 14 industrial estates drain their untreated effluents into this river. The agricultural drained water is another source of pollution and this river water is rich in heavy metals, pesticide residues, phenols, oils, grease, alkalis and acids [11]. The self-purifying property of river water is unable to clear the pollution, and the polluted water poses a serious risk to public health especially in areas where river water is used for irrigation.

Keeping this in view of the Musi river pollution and its direct or indirect effect on environment, animal and human system, a study was conducted to analyze the milk samples on the banks of river Musi for the presence of pesticide residues. The study has been conducted on river Musi, Located in Telangana, India.

2. Materials and Methods

2.1 Collection of samples

This study was based on 48 milk samples collected from six divided zones (8 from each zone) (Table-1) on the downstream of Musi river belt, Telangana, India in 2013. Zones were divided based on earlier reports on Musi river pollution by Pullaiah, 2013 [11]. Sterilized glass bottles were used to collect 250 ml milk samples, labeled and transported to lab in ice pack, they were kept at 4°C until analysis. Samples were subjected to analysis within 24 h from their arrival.

Table 1: Selected zones and covered areas along the Musi river belt, Telangana, India

I	**Attapur, Langer House, Upper pally, Kishan Bagh, Bahadurpura, Puranapool, Budvel, High court.**
II	Chadhar ghat, Malakpet, Morarambagh, Golnaka, Amberpet, Ramanthapur, Nagole, Uppal.
III	Peerzadiguda, K. singaram, Thimaiguda, Pratapa singaram, Korremulla, Bacharam, Bandaraviral, Chinna raviralla.
IV	Pillai Palli, Rudravelly, Brahmanapally, Venkiryala, Edulabad, Nadama Khada, Shivareddy gudem, Alinagar.
V	Indriyala, D.R.palli, Wankamamidi, Shaligowraram, Dharmaram, Chittur, Jajireddygudem, Manimadde.
VI	Musi reservoir, Yendlapally, Kasarabad, Beemavaram, Dasaphad, M. gudem, Irkigudem, Wazirabad.

2.2 Pesticides analyzed

The residues of certain pesticides of organophosphates (Dichlorovas, Phorate, Methyl parathion, Fenitrotion, Quinolphos, Profenphos, Ethion, Phosalone, Lambda Cyhalotrin) in milk samples collected from six zones of Musi river belt area.

2.3 Chemicals and reagents

Acetonitrile, acetone, dichloromethane, graphitized carbon black, hexane, magnesium sulfate, silica gel, sodium chloride, sodium sulfate, prostate specific antigen (PSA) of high-performance liquid chromatography residue grade obtained from Qualigens and Merck specialties Pvt. Ltd. Analytical standards with >99% purity were obtained from Dr. Ehrenstorfer, Germany during 2012 and stored in deep free maintained at −40°C.

2.4 Method validation

The required quantity of (Organophosphorus) international standards prepared from certified reference materials were added to each 15 g sample to get fortification levels of 0.05 ppm and 0.1 ppm in three replications each. The AOAC official method 2007.01 with slight modifications was validated for the estimation of the limit of quantification (LOQ) of organophosphorus in milk. Milk samples, 5 g of Milk was taken into 250 ml beaker and 20 g of silica gel and 20 g of anhydrous sodium sulfate was added. Glass column was prepared with 40 ml of dichloromethane over cotton plug, sample was made into slurry with dichloromethane then this was transfer to column and allowed to stand for 90 min then dichloromethane was eluted drop wise, again the sample column was eluted with a mixture of 150 ml acetone: Dichloromethane (2:1 v/w) and anhydrous sodium sulfate was added to the elute, then concentrated to 2-3 ml, 10-15 ml of hexane was added to the concentrate to remove dichloromethane completely, volume was made with n-hexane. Finally, an aliquot of each extract was transferred to 2 ml injection vials to be ready for the analysis.

A Schimadzu 2010 gas chromatography (GC) equipment with a VF-1MS capillary column and with electron capture detector (ECD) and flame photometric detector. All the chemicals were purchased from M/s. Merck specialties pvt. Limited and were pesticide residue grade and all pesticide residue standards were purchased from Dr. Erhenstorfer, Germany during 2012. The gas chromatographic analysis was performed under the following conditions (Table-2). A volume of 1 ml sample was injected into the GC; peaks were identified by comparing their retention times with those of standards under the same injection conditions. The peak areas of the various peaks whose retention times coincide with the standards were extracted on their corresponding calibration curves to obtain the concentrations.

Table 2: Details of GC operating parameters.

GC	**GC-Schimadzu 2010**
Column	VF-1ms capillary column 30 m length, 0.25 mm internal diameter, 0.25 mm film thickness; 1% methyl siloxane
Column oven (°C)	260 (isothermal)
Detectors	ECD FPD
Detector temperature (°C)	280
Injector temperature (°C)	260
Injector status	Front injector type 1177 split/splitless Split ratio: 1:5
Carrier gas	Nitrogen, Iolar II, Purity 99.99%
Carrier gas flow (ml min^{-1})	1 ml/min
Make-up flow (ml min^{-1})	35 ml/min
Total run time (min)	60 min
ECD=Electron capture detector, FPD=Flame photometric detector, GC=Gas chromatography	

Retention time	ECD	PFPD
Dichlorovas	3.947	3.889
Phorate	13.523	13.427
Methyl parathion	20.249	20.090
Fenitrothion	-	21.726
Quinolphos	26.775	26.587
Profenphos	30.796	30.606
Ethion	34.634	34.436
Phosalone	47.747	47.511
λ - Cyhalotrin	48.497	-
ECD=Electron capture detector, PFPD=Pulsated flame photometric detector		

3. Results and Discussion

A total of 48 milk samples collected from all the six zones of Musi river belt and were analyzed for OPPs residues. Concentration of various residues in each sample was calculated (in mg/kg sample). In the present study, the average recoveries of OPPs in milk were from 91.25% at 0.05 ppm and 86.77% at 0.1 ppm. The efficiency of extraction methodologies were evaluated based on the recoveries of residues, and a recovery of 75-102% is considered as acceptable [12]. Hence, the extraction procedures employed in these experiments were efficient in recovering the maximum amount of residues present in the samples. The elute pattern of various OPPs (0.05 ppm) along with specific retention time are depicted in Figure-1 for Electron Capture Detector (ECD) and Figure-2 for pulsated flame photometric detector (PFPD). The limit of detection and Limit of quantification for OPPS was 0.05 ppm and 0.05 ppm respectively for both ECD and PFPD.

Milk samples collected from zone IV contain the residual concentration of Quinolphos 0.06 ± 0.015 (0.04-0.08) ppm, (Figure-3), Other Organophosphorus compounds collected from zone I, II, III, IV, V and VI were below detection limit in all other milk samples in the present study. Similar studies were conducted by Astha Pandey *et al.* [13] for Residual levels of Dichlorovas, in Buffalo Milk Results showed that 40 % of the milk samples were contaminated with pesticides residues of organophosphorous. Studies were conducted by by Pagliuca. *et. al.* [14], for phorate residues in milk samples collected in Italian dairy plants where in present study Phorate residues could not be detected. Residues of Methyl parathion have been recorded in many foods, even occasionally in milk [15] and a percentage of 0.83% of methyl parathion in milk samples were detected by Melgar *et. al.,* [16]. In a study on detection of residual levels of fenitrotion Lactating dairy cows were fed 50 mg/kg of fenitrothion (dry weight basis) in the feed for 29 days. No residues of fenitrothion appeared in milk by Snelson *et. al.,* [17] and in another study Silage prepared from corn treated at up to 3.36 kg/ha of fenitrothion was fed to lactating dairy cattle for 8 weeks. Fenitrothion was not detected in the milk [18]. Milk samples collected from zone IV contains the residual concentration of quinolphos of 0.06 ± 0.015 (0.04-0.08) ppm, Similar studies were done in milk samples for quinolphos residues, collected from milkfed depots in Ludhiana [19], whereas profenofos were detected at the mean levels; 1.96 ± 0.3 µg/kg in buffalo milk [20] but in the present study profenofos residues were not detected. In a study, gas chromatographic analysis of pesticide residues in bovine milk (n = 312) from Punjab, India, showed cyhalothrin and ethion was reported in milk samples [21] whereas in the present study cyhalotrin and Ethion were not detected. Similar results as of present study were detected by Rafael *et. al.,* [22] for phosalone. (Table-3).

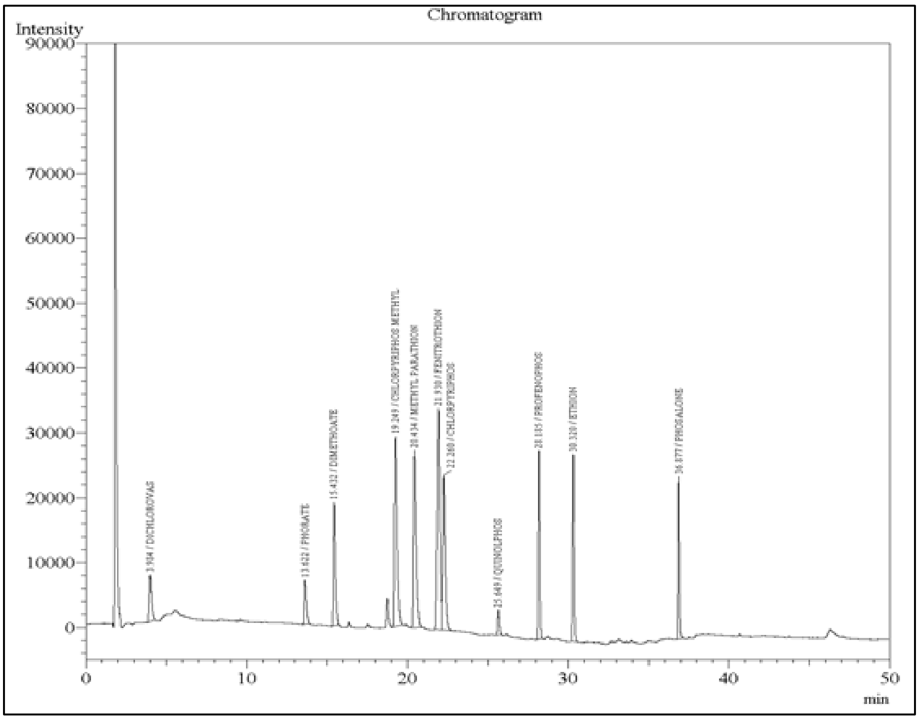

Fig 1: Elution pattern of organophosphorus pesticide standards mixture by electron capture detector.

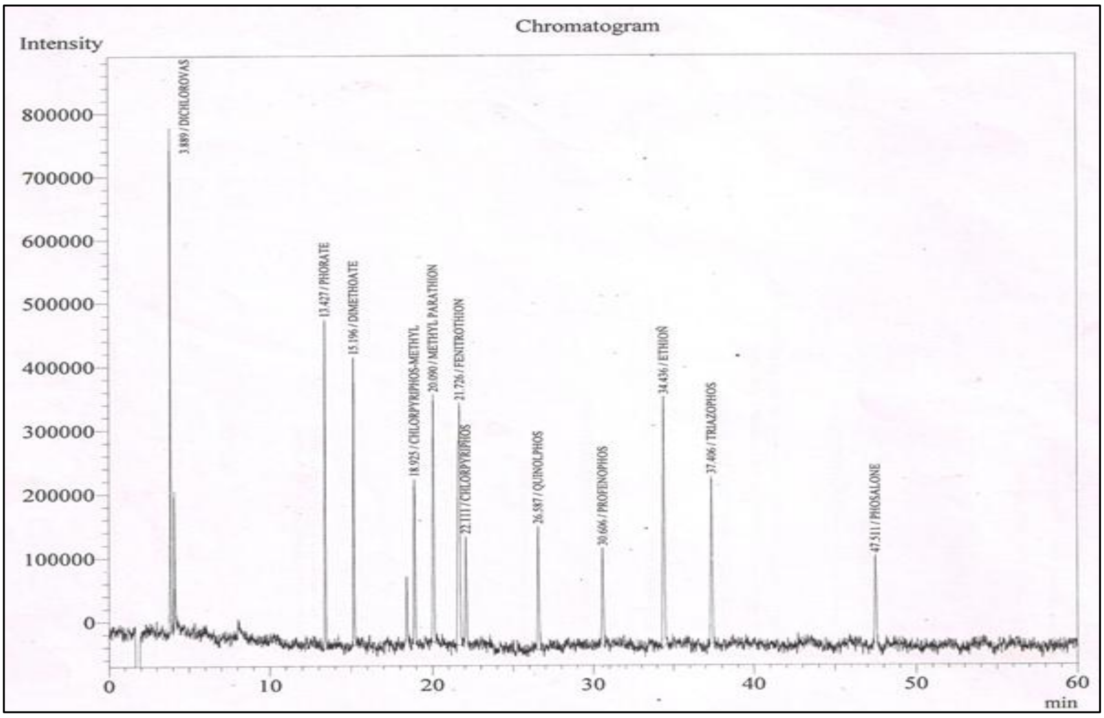

Fig 2: Elution pattern of organophosphorus pesticide standards mixture by pulsated flame photometric detector.

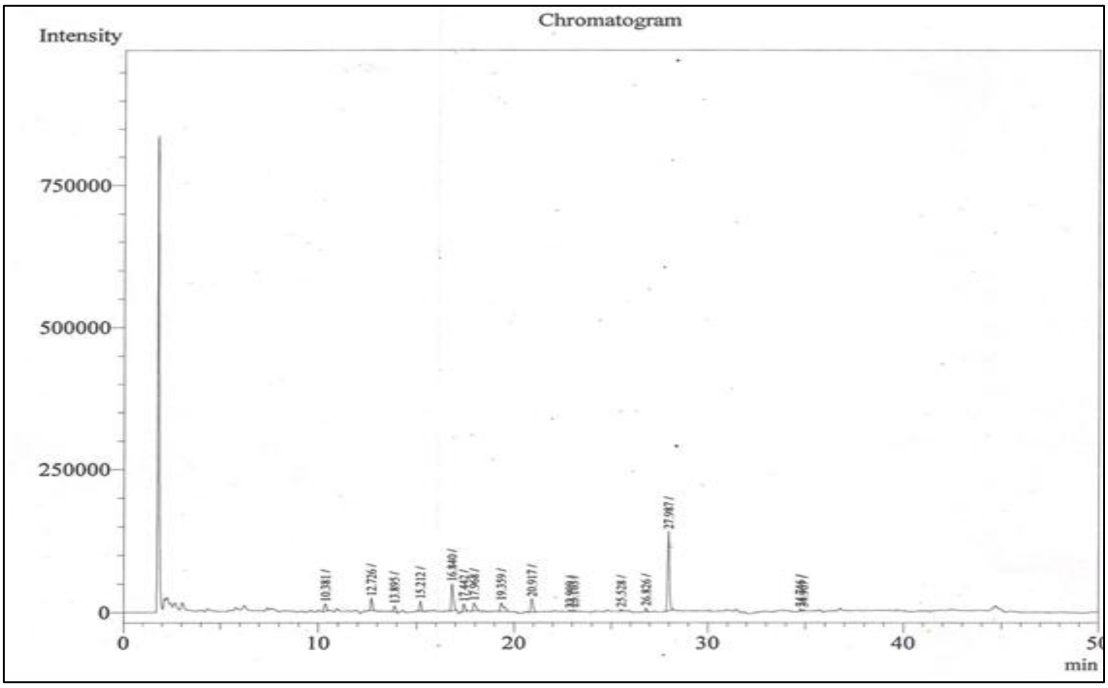

Fig 3: Elution pattern of organophosphorus pesticide residues in milk samples from zone IV of the Musi river belt.

Table 3: Mean residual levels (ppm) of organophosphorus pesticides in Milk samples along Musi River.

Samples	Zones	Dichlorovas	Phorate	Methyl parathion	Fenitrothion	Quinolphos	Profenphos	Ethion	Phosalone	Lambda Cyhalothrin
Milk	Zone I	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Zone II	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Zone III	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Zone IV	BDL	BDL	BDL	BDL	0.06	BDL	BDL	BDL	BDL
	Zone V	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Zone VI	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

(Each value is mean of 8 replications), Zone I: Attapur, Langer House, Upper pally, Kishan Bagh, Bahadurpura, Puranapool, Budvel, High court, Zone II: Chadhar ghat, Malakpet, Morarambagh, Golnaka, Amberpet, Ramanthapur, Nagole, Uppal, Zone III: Peerzadiguda, K. singaram, Thimaiguda, Pratapa singaram, Korremulla, Bacharam, Bandaraviral, Chinna raviralla, Zone IV: Pillai Palli, Rudravelly, Brahmanapally, Venkiryala, Edulabad, Nadama Khada, Shivareddy gudem, Alinagar, Zone V: Indriyala, D.R.palli, Wankamamidi, Shaligowaram, Dharmaram, Chittur, Jajireddygudem, Manimadde, Zone VI: Musi reservoir, Yendlapally, Kasarabad, Beemavaram, Dasaphad, M.gudem, Irkigudem, Wazirabad, BDL=Below determination level (<0.05)

4. Conclusion

From this study, it can be concluded that all the pesticide residues in milk samples were below the MRL except Quinolphos in milk were slightly above the MRL values specified by European Union (EU) it might be due to use of these pesticides on vegetable crops grown on the banks of Musi river belt. The results of OPPs in different samples were detected by ECD and confirmed by PFPD. Further work is needed to determine the bioaccumulation of these toxic elements in the food web and the associated risks to the ecosystem and human health.

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6. References

1. Kampire E, Kiremire BT, Nyanzi SA, Kishimba M. Organochlorine pesticide in fresh and pasteurized cow’s milk from Kampala markets. Chemosphere. 2011; 84(7):923-927.
2. Fagnani R, Beloti V, Battaglini APP, Dunga KS, Tamanini R. Organophosphorus and carbamates residues in milk and feedstuff supplied to dairy cattle. Pesq Vet Bras. 2011; 31(7):598-602.
3. Johansen P, Muir D, Asmund G, Riget F. Human exposure to contaminants in the traditional Greenland diet. Sci Total Environ. 2004; 331(1-3):189-206.
4. Schecter A, Cramer P, Boggess K, Stanley J, Olson JR. Levels of dioxins, dibenzofurans, PCB and DDE congeners in pooled food samples collected in 1995 at supermarkets across the United States. Chemosphere. 1997; 34(5-7):1437-1447.
5. Fox GA, Kennedy SW, Norstrom RJ, Wigfield DC. Porphyria in herring gulls: a biochemical response to chemical contamination of Great Lakes food chains. Environ. Toxicol Chem. 1988; 7(10):831-839.
6. Safe S. Polychlorinated biphenyls (PCBs), dibenzo-pdioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Critical Rev Toxicol. 1990; 21(1):51-88.
7. Tsiplakou E, Anagnostopoulos CJ, Liapis K, Haroutounian SA, Zervas G. Pesticides residues in milks and feedstuff of farm animals drawn from Greece. Chemosphere. 2010; 80:504-512.
8. Jahed Khaniki Gh R. Chemical contaminants in milk and public health concerns: A review. Int. J Dairy Sci. 2007; 2:104-115.
9. Mathur S C. Pesticides industry in India, Pestic Inf. 1993; 19:7-15.
10. Tsiplakou E, Anagnostopoulos CT, Liapis K, Haroutounian SA, Zervas G. Pesticides residues in milk and feed stuff of farm animals drawn from greece. Chemosphere. 2010; 80(5):504-512.
11. Cheepi P. Assessing the economic impact of water pollution – A case study of musi river Hyderabad, India. Int. Res. J Soc. Sci. 2013; 2(1):18-23.
12. Solymos M, Visi E, Karoly G, Beke Berezi B, Gyorfi L. Comparison of extraction methods to monitor pesticide residues in surface water. J Chromatogr. Sci. 2001; 39(8):325-331.
13. Astha Pandey *et al*. International Journal of ChemTech Research. 2017; 10(1):231-238.
14. Pagliuca G, Serraino A, Gazzotti T, Zironi E, Borsari A, Rosmini R. J Dairy Res. 2006; 73:340. https://doi.org/10.1017/S002202990600169
15. ATSDR (Agency for Toxic Substances and Disease Registry). Toxicological Profile for Methyl Parathion. Agency for Toxic Substances and Disease Registry. Atlanta USA. 2001. http://www. atsdr. cdc. gov. toxprofiles/tp48.html, accessed on 15/11/2012.
16. Melgar MJ, Santaeufemia M, García M. Organophosphorus pesticide residues in raw milk and infant formulas from Spanish northwest, Journal of Environmental Science and Health, Part B. 2010; 45(7):595-600. DOI: 10.1080/03601234.2010.502394
17. Snelson J The Fate of Fenitrothion Residues on Wheat Gluten Following Incorporation into Bread. 1979; 91-0051.
18. Sumithion Technical Manual (undated), Sumitomo Chemical Co. Ltd.
19. Neelam Richhariya, Sudeep Mishra, Lalitesh Kumar Thakur, Rachana Rani. Pesticide residues contamination of liquid milk in India- A review. World Journal of Pharmacy and Pharmaceutical Sciences. 6(4):549-562. DOI: 10.20959/wjpps20174-8918
20. Abbassy MMS. Pesticide Residues in Buffalo and Human Breast Milk of Vegetables and Fruits Farming Community at Northern of Delta in Egypt. J Environ Anal Toxicol. 2017; 7:432. doi: 10.4172/2161-0525.1000432
21. Bedi JS, Gill JP, Aulakh RS, Kaur P. Pesticide Residues in Bovine Milk in Punjab, India: Spatial Variation and Risk Assessment to Human Health. Arch Environ Contam Toxicol. 2015; 69(2):230-40. doi: 10.1007/s00244-015-0163-6.
22. Rafael Fagnani, Vanerli Beloti, Ana Paula P, Battaglini, Karen da S, Dunga, Ronaldo Tamanini. Organophosphorus and carbamates residues in milk and feedstuff supplied to dairy cattle. Pesq. Vet. Bras. 2011; 31:7. Rio de Janeiro http://dx.doi.org/10.1590/S0100-736X2011000700009.