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ESTIMATION OF PESTICIDE RESIDUES IN HUMAN BLOOD SAMPLES OF OCCUPATIONAL AND NON-OCCUPATIONAL RESIDENTS OF RAJASTHAN

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ABSTRACT

The aim of the present study was to evaluate the pesticide residues in human blood samples of volunteers related to Jaipur, Bhilwara and Ajmer districts of Rajasthan. The volunteers were categorized into two main groups on the basis of gender and age. Group I was subcategorized as male and female and Group II was subcategorized according to their age: 1 to 3, 20 to 30 and 50 to 70 yrs. Qualitative analysis of pesticide residues in blood samples were carried out by using thin layer chromatography. 33% samples were found to be positive regarding presence of pesticide residues in their respective blood samples out of the total samples analysed by thin layer chromatography. In the present study qualitative and quantitative estimation of pesticide residues were carried out by gas chromatography-Mass spectrometry. The blood samples of 15 volunteers were analysed for pesticide residues using GC-Mass spectrometry. 29 % of female volunteers were found positive for presence of pesticide residues in their blood samples whereas 62 % of samples of male volunteers were found positive for the presence of pesticide residues in the blood samples analysed by GC-Mass spectrometry. Out of 5 blood samples of infants analysed for pesticides residues, Deltamethrin was detected in one of the sample (0.18mg / ml). 40% of the male volunteers belonging to age between 30 to 35 years were found to contain residues of chlorpyrifos in their blood samples with concentrations range of 0.002836- 0.241210 µg /ml. Cypermethrin was also detected in blood samples of 35% of male volunteers with the concentration range of 0.344841 - 0.543612 µg /ml. The residues of methyl carbamate was detected in 20% of the female volunteers of age belonging to 50-55 years with the concentration range of 0.003345 - 0.005632 µg/ml. The quantity of pesticide residues detected in some blood samples of agro-professionals were found to be at the alarming level.

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INTRODUCTION

In India, pesticides are one of the most essential components of modern agricultural technology and have contributed greatly in the increase of agriculture yields and control of vector-borne diseases. The environmental conditions in tropical countries are highly conducive to rapid multiplication of pests. Therefore, a wide variety of pesticides is used in tropical countries to combat these crop pests and disease vectors [1]. There are more than 234 registered pesticides in India and the Indian pesticide industry includes more than 125 large and medium scale producers of more than 500 pesticide products.[2] In India, utilization of pesticides are not well controlled as compared to the developed countries due to ineffective legislation and deficiency in awareness. Thus, due to mishandling and malpractices as the spray workers do not follow the necessary

precautions, dangerous consequences may occur in their own health and causing various troubles such as pesticide contamination to the food chain and pesticide residue accumulation in the consumer's body [3]. Intensive use of pesticides for agriculture and health purposes in developing countries, has led to widespread pollution of the environment [4-9]. Owing to their properties, including low volatility, chemical stability, environmental resistance, lipophilicity and slow metabolic degradation, bio accumulation and bio concentration has occurred in birds and mammals and the food chain [10]. Contamination has also been demonstrated in humans. Measurable levels of organochlorine pesticides have been found in human adipose tissues, blood and breast milk throughout the world [11-13].

Nowadays an extensive attention is paid by FAO (Food and Agricultural Organization and WHO (World Health

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Organization) on pesticide residues in food, environment, and the possibility of harm to human following long term of low levels exposure and consequent effects of chronic poisoning. Human beings can be exposed to pesticides either by occupational (manufacturing/formulation of pesticides and during application in the agricultural fields) or nonoccupational routes (pollution of the ecosystem through food chain). Farm workers come into direct contact with pesticides at work as well and are occupationally exposed to them [14]. In agricultural environment the spray-workers and their families can absorb a measurable quantity of pesticides; therefore most research concerns have focused on the acute or life threatening effects. When a person is exposed to pesticides, body's detoxification mechanisms are activated. Some pesticides are metabolized into different chemicals and excreted and some are stored in fatty tissues in the body. Biological monitoring provides the basis for estimating an internal chemical dose by measuring pesticide and their metabolite compound concentrations in selected tissues, fluids, or bodily waste (feces and or urine) [15]. The determination of serum levels of pesticides can be used as a biomarker of exposure for evaluating the health effects at certain levels [16]. Body burden data from analysis of blood provides evidence of exposure to chemicals stored in our body. Analysis of blood provides evidence of exposure of the body to pesticides and gives an indication of the body burden of the pesticide residues. Monitoring of organochlorine pesticide concentration in blood is most appropriate because these pesticides are lipophilic in nature. Similarly, monitoring of organophosphate pesticide concentrations in blood or blood products (serum, plasma) offers several advantages. The parent compounds can be monitored directly in blood products instead of their metabolites, which are usually measured in urine. Thus, the present study was planned to estimate pesticide residues in blood samples of some volunteers of selected rural areas who were either directly or indirectly exposed to pesticides.

The objective of this study is to analyze the pesticide residues in blood samples of human beings belonging to different age groups of various regions in Rajasthan employing chromatographic and spectral techniques.

MATERIAL AND METHODS

Study Areas

This study was carried out on volunteers who were occupationally spray-workers and non occupational inhabitants of Jaipur, Ajmer and Bhilwara.

Collection of Blood Sample

The 5 ml of blood was collected in residue free heparinised vials with the help of sterilized syringe. The samples were kept in a plastic container and stored at 5°C until analysis.

The volunteers were categorized into two main groups on the basis of gender and age. Group I was subcategorised as male and female and Group II was subcategorised according to their age: 1 to 3, 20 to 30 and 50 to 70 yrs.

Pesticide Extraction from Blood

Pesticide residues from blood samples were extracted in hexane [17].

Analysis of Blood Samples

The analysis includes qualitative and quantitative estimation of pesticide residues with the help of chromatography and spectral techniques.

Qualitative Analysis of Pesticide Residues Extracted From Human Blood Samples

Qualitative analysis of pesticide residues in blood samples were carried out by using thin layer chromatography [18].

MATERIALS

Equipment: TLC aluminium sheet silica gel 60 F254, Merck KGaA, Germany and glass chromatographic chamber (Madhurai, India) were used for the experiment. A glass chamber of suitable size with an airtight lid was equilibrated with respective solvent system for 20-30 min prior to each experiment. For each of the solvent systems separate cleaned chamber was used. Fine glass capillaries were used for spotting the samples on TLC plates.

All chemicals used in the present investigation were of analytical grade. All the solvents were dried and then distilled out. Standards of pesticides were purchased from Sigma Aldrich (Germany).

Spray reagents

Visualization reagents used in this study are

- I. 1% Diphenylamine solution in ethanol
- II. 0.5% palladium chloride solution
- III. 1% Ammonical Silver Nitrate solution
- IV. 1 % Zinc chloride and 0.5 % Diphenyl amine in acetone [19]

Mobile phase

Solvent system used for the development of TLC plates was hexane: acetone (80:20).

Procedure

The extracted sample from blood along with the standard sample was loaded on precoated TLC plates. The spots are allowed to dry and then spotted plates were developed with solvent system Hexane: Acetone(80:20). After development TLC plates were taken out of the solvent chamber and dried in air. Each sample was spotted on two plates so that simultaneously two types of spray reagent can be used for detection of compound. After spraying the chromogenic reagent on each of the TLC plates, the respective coloured spots were observed. The spot can be identified by comparing R_f value of samples with R_f value of control.

Observation

33% samples were found to be positive regarding presence of pesticide residues in their respective blood samples out of the total samples analysed by thin layer chromatography. Black brown colour spots appeared on TLC plates after spraying with ammonical silver nitrate solution followed by exposure of UV

light as shown in Figure 1. This confirms the presence of organochlorine pesticide in blood samples.

Appearance of purple colour spots after spraying with zinc chloride and diphenylamine solutions as shown in Figure 2 was due to the presence of organophosphorous pesticide in blood samples. Green blue colour appear on TLC plates after spraying with 1% diphenylamine solution followed by exposure of UV light at 366 nm as shown in Fig. 3 was due to the presence of organochlorine pesticides. Appearance of yellow colour spots surrounded by brown colour on white background after spraying with palladium chloride solution as shown in Fig 4 was due to presence of organophosphorus pesticide in blood sample.

Following Figures exhibit the different coloured spots of pesticide residues after development of TLC plates in Hexane: Acetone (80:20) solvent system.



Fig.1 Brown colour spots on TLC plates after spraying by ammonical silver nitrate solution followed by exposure of UV light



Fig.2 Purple blue colour spot are appeared on TLC plates after spraying with zinc chloride and diphenylamine

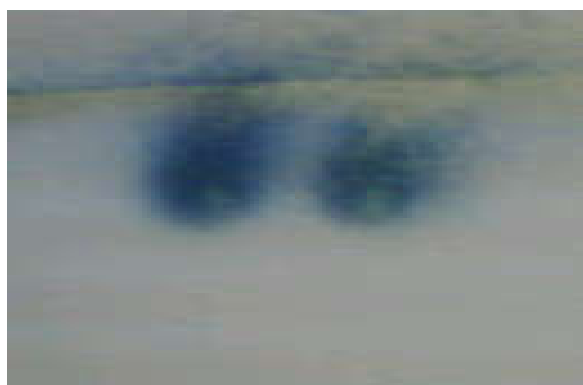


Fig.3 Green blue colour spots appeared on TLC plates after spraying with diphenylamine solution followed by UV exposure of 366nm.



Fig.4 Yellow colour spots appeared on TLC plates after spraying with palladium chloride

RESULTS AND DISCUSSION

33% samples were found to be positive regarding presence of pesticide residues in their respective blood samples out of the total samples analysed by thin layer chromatography. Fig 5 illustrates the study of identification of pesticide residues in blood samples of volunteers categorised gender wise as group I. It is evident that 26 % of female volunteers were found positive for the presence of pesticide residues in their blood samples whereas 40 % of samples of the male volunteers are found positive for pesticide residues in the blood samples. The variation in gender wise obtained data may be due to the difference in time of exposure. Male volunteers are more vulnerable because they are working in fields comparatively more than female candidates. The another reason is that it is believed that lactation and menstruation are the most efficient means of reducing a women’s body burden of pesticide residues [20].

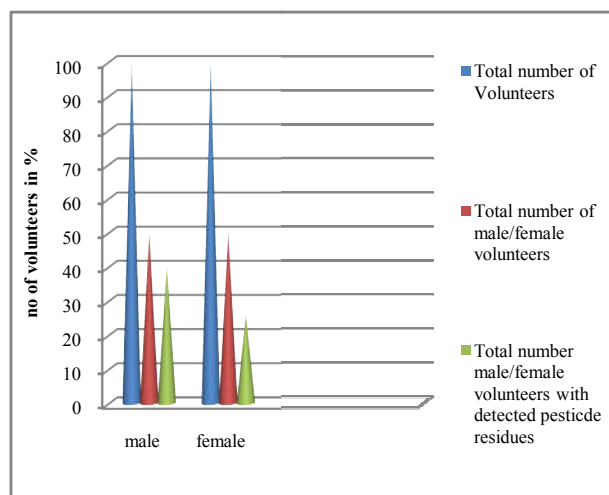


Fig.5. Statistical analysis of volunteers (group I) for the presence of pesticide residues in their respective blood samples identified by thin layer chromatography

Figure 6.(a) & (b) shows the statistical analysis of blood samples of volunteers belonging to different age groups categorised as group II. It is evident from the figure that comparatively high percentage (46.6%) of volunteers belonging to the age group of 20-40 years was found positive

for the presence of pesticide residues in their blood samples than the volunteers belonging to the age group of 50-70 years (20 %). The higher percentage of younger volunteers may be attributed due to more exposure time in the field during handling and spraying of pesticides.

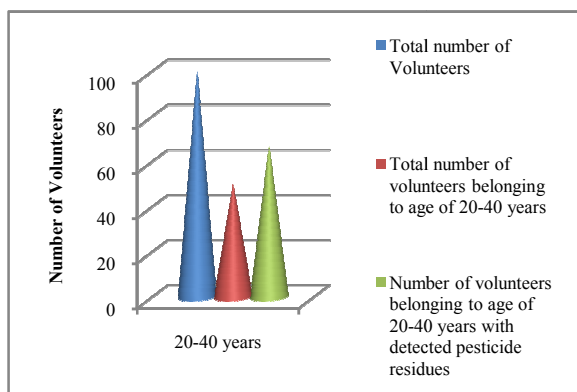


Fig.6(a) Statistical analysis of volunteers (group II) belonging to age groups 20-40 for the presence of pesticide residues in their respective blood samples identified by thin layer chromatography.

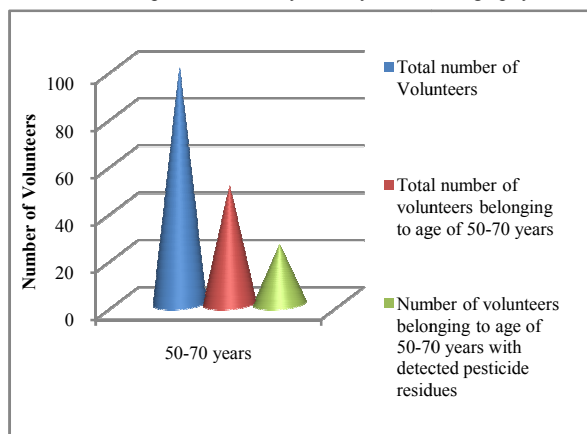


Fig.6.(b) Statistical analysis of volunteers (group II) belonging to age groups 50-70yrs for the presence of pesticide residues in their respective blood samples identified by thin layer chromatography

Quantitative Estimation of Pesticide Residues in Blood Samples

Gas chromatography (GC) method is suitable for the separation and quantitative estimation of compounds which are volatile or semi-volatile and thermally stable at the temperature of the measurement. In the present study qualitative and quantitative estimation of pesticide residues were carried out by gas chromatography-Mass spectrometry [21-22]. The blood samples collected were categorized as follows: Group I (Gender wise) and Group II (Age wise 1-3 yrs, 20-40 yrs and 50-70 yrs). The extracted samples were subjected to GC-Mass spectrometry for further analysis.

Instrumentation

Quantitative estimation of pesticide residues present in the extracts was carried out employing GC-MS of Hewlett-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with using Agilent 7890A/5975C GC HP-5. Separations were performed on a capillary column (phenyl methyl siloxane, 30 m x 0.25 mm i.d.; film thickness, 0.25 µm).

Instrumental Conditions

For Gas chromatography-Mass spectrometry the working condition were as follows: Helium (He) was used as the carrier gas with split ratio 1:5 at 0.9 mL/min flow rate. Oven temperature was programmed as 100 °C to 280°C at 1 to 40 °C/min; detector temperature was 250 to 280°C. The volume of injection was 0.60 µl. The components of the samples were identified by comparison of their mass spectra and retention time with standard samples. The spectra recorded are shown in Figure 7 to Figure 12.

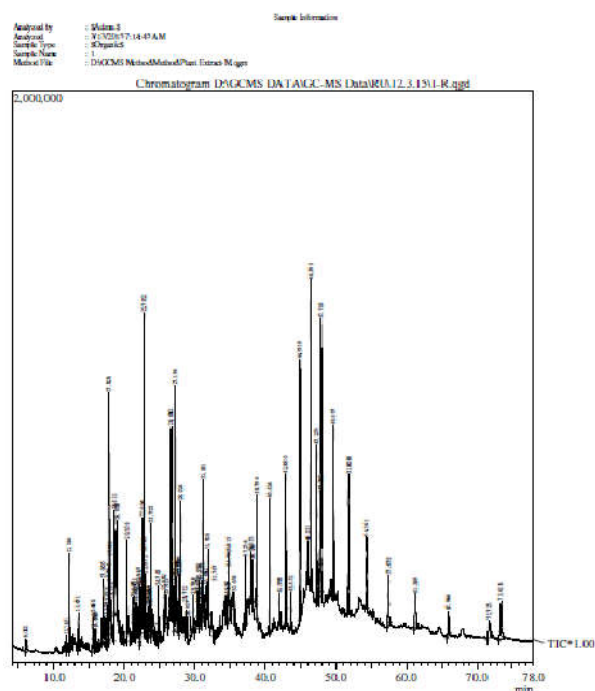


Figure 7 GC-Mass spectra of blood sample

Data File:	SAS2	Injection Volume(µl):	1.50
Sample ID:	Samrml	Acquisition Date:	04/16/15 06:01:27 PM
Run Time(min):	36.44	Comments:	
Scan:	10713	Low Mass(m/z):	50
High Mass(m/z):	700	Instrument Name:	TSQ 8000

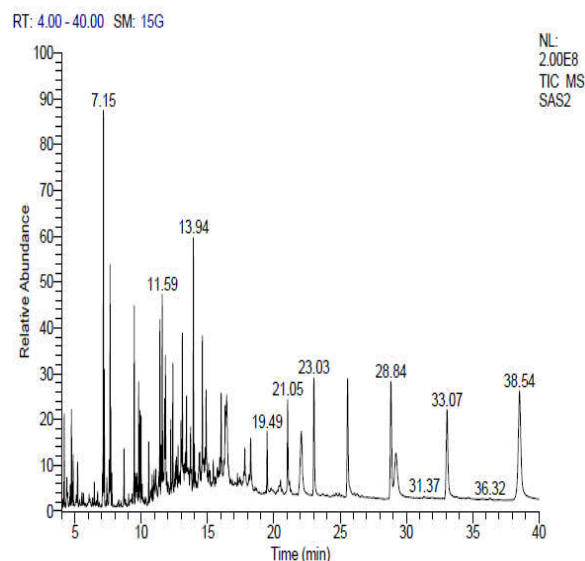


Figure 8 GC-Mass spectra of blood sample

Data File: SAS_05
 Sample ID: A5
 Run Time(min): 58.10
 Scans: 17081
 High Mass(m/z): 1000
 Injection Volume(µl): 0.60
 Acquisition Date: 04/21/15 02:23:40 PM
 Comments:
 Low Mass(m/z): 50
 Instrument Name: TSQ 8000

RT: 4.00 - 60.00 SM: 15G

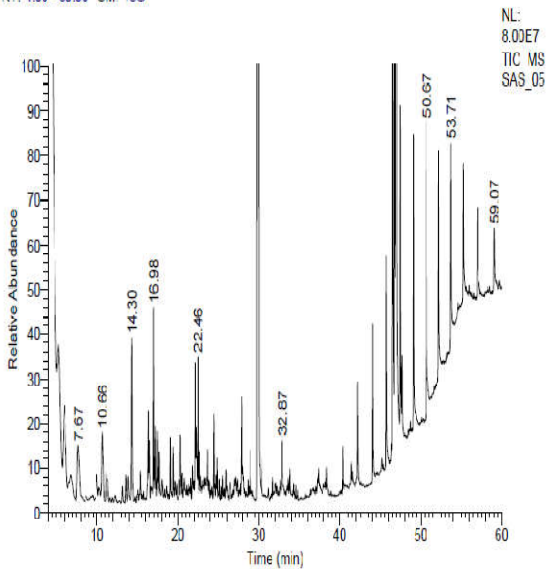


Figure 9 GC-Mass spectra of blood sample

Data File: SAS_10
 Sample ID: A10
 Run Time(min): 58.11
 Scans: 17085
 High Mass(m/z): 1000
 Injection Volume(µl): 0.60
 Acquisition Date: 04/21/15 07:53:03 PM
 Comments:
 Low Mass(m/z): 50
 Instrument Name: TSQ 8000

RT: 5.00 - 60.00 SM: 15G

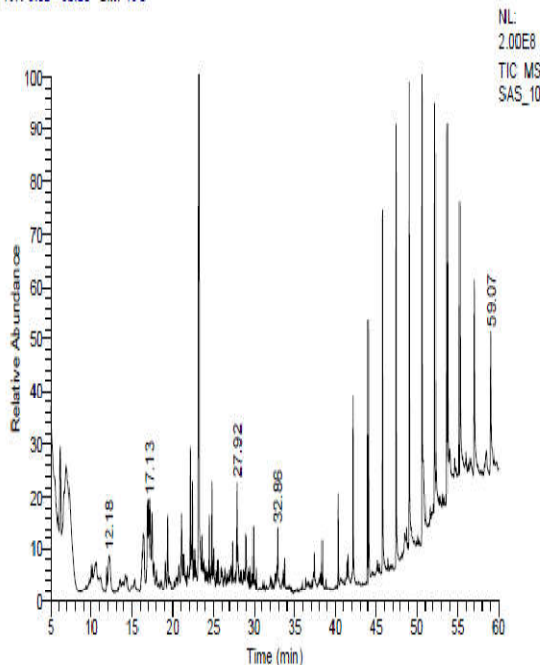


Figure 10 GC-Mass spectra of blood sample

Data File: SAS_08
 Sample ID: A8
 Run Time(min): 58.12
 Scans: 17089
 High Mass(m/z): 1000
 Injection Volume(µl): 0.60
 Acquisition Date: 04/21/15 05:41:22 PM
 Comments:
 Low Mass(m/z): 50
 Instrument Name: TSQ 8000

RT: 4.00 - 60.00 SM: 15G

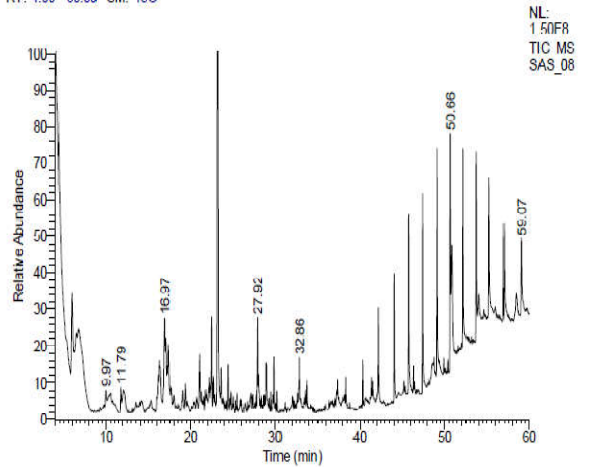


Figure 11 GC-Mass spectra of blood sample

Data File: SAS_10
 Sample ID: A10
 Run Time(min): 58.11
 Scans: 17085
 High Mass(m/z): 1000
 Injection Volume(µl): 0.60
 Acquisition Date: 04/21/15 07:53:03 PM
 Comments:
 Low Mass(m/z): 50
 Instrument Name: TSQ 8000

RT: 5.00 - 60.00 SM: 15G

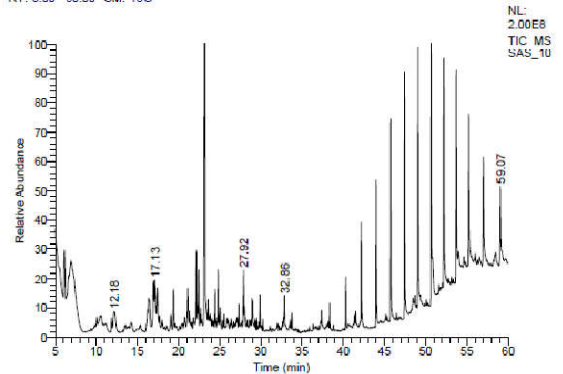


Figure 12 GC-Mass spectra of blood sample

RESULTS AND DISCUSSION

The blood samples of 15 volunteers were analysed for pesticide residues using GC-Mass spectrometry. The volunteers were categorized into two groups viz. Gender wise and age wise.

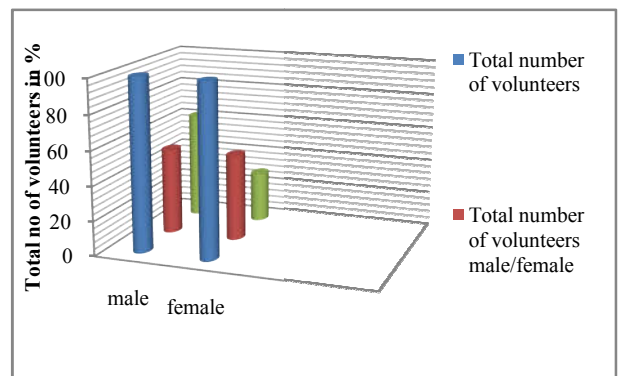


Figure 13 Statistical analysis of volunteers belonging to group I (genderwise) for the presence of pesticide residues in their respective blood samples studied by GC-Mass spectrometry.

It is evident from Fig 13 that 29 % of female volunteers were found positive for presence of pesticide residues in their blood samples whereas 62 % of samples of male volunteers were found positive for the presence of pesticide residues in the blood samples analysed by GC-Mass spectrometry.

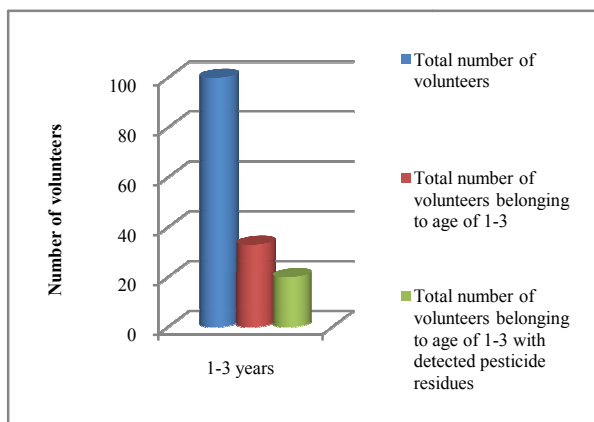


Fig.14 a Statistical analysis of volunteers (group II) belonging to age groups 1-3 yrs for the presence of pesticide residues in their respective blood samples analysed by GC-Mass spectrometry.

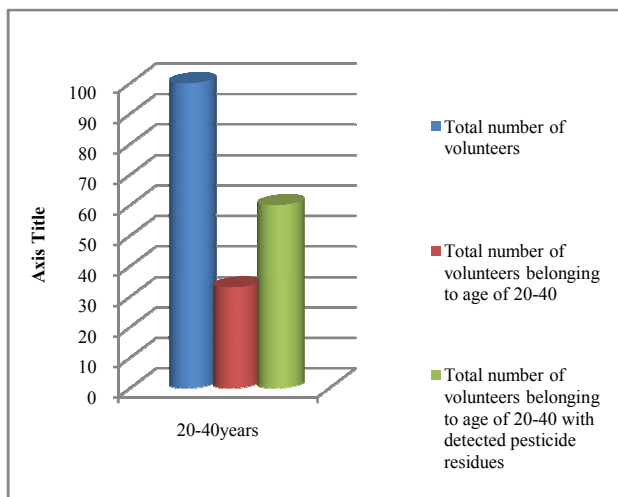


Fig.14 b Statistical analysis of volunteers (group II) belonging to age groups 20-40yrs for the presence of pesticide residues in their respective blood samples analysed by GC-Mass spectrometry.

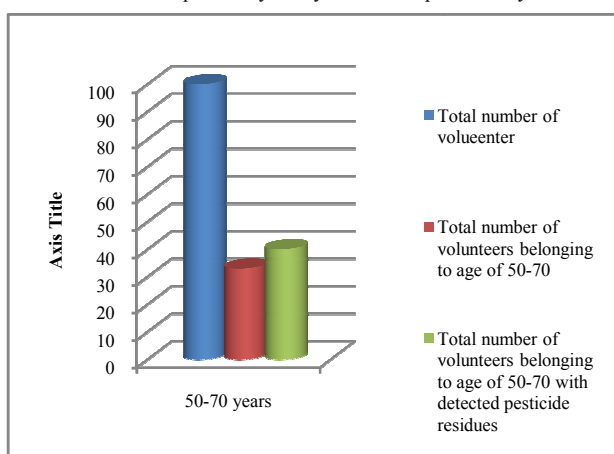


Fig.14 c Statistical analysis of volunteers (group II) belonging to age groups 50-70yrs for the presence of pesticide residues in their respective blood samples analysed by GC-Mass spectrometry.

The inequality of obtained data is due to the variation in time of exposure. The another reason is that it is believed that lactation and menstruation are the most efficient means of reducing a women's body burden of pesticide residues [20].

It is evident from figure 14(a,b & c) that a comparatively high percentage (60%) was found in volunteers belonging to the age group of 20-40 years who have detected residues in their blood samples than the volunteers belonging to the age group of 50-70 years(40%). Minimum percentage (20%) was found in the samples of age group 1-3 years.

- Out of 5 blood samples of infants analysed for pesticides residues, Deltamethrin was detected in one of the sample (0.18mg / ml). Several literature studies indicate that infants and children might be more susceptible to deltamethrin toxicity as a result of post-natal exposure. The enzymes accounting for the metabolism of deltamethrin either are not present in the same levels in children as they are in adults, or they are not as active in children. Similarly, enzyme profiles in infants and children are less developed than they are in adults and can be highly variable in quantity and activity, especially in infants. Until sufficient evidence is available demonstrating that infants and children have the capacity to metabolize deltamethrin in a manner comparable to the way in which adults metabolize it, quantitative susceptibility is being assumed [23-24].
- 40% of the male volunteers belonging to age between 30 to 35 years were found to contain residues of chlorpyrifos in their blood samples with concentrations range of 0.002836- 0.241210 µg /ml.
- Cypermethrin was also detected in blood samples of 35% of male volunteers with the concentration range of 0.344841 -0.543612 µg /ml.
- The residues of methyl carbamate was detected in 20% of the female volunteers of age belonging to 50-55 years with the concentration range of 0.003345 - 0.005632 µg/ml.
- Dimethoate was also detected in 10% of male volunteers blood sample with the mean value concentration of 0.01051µg/ml.

CONCLUSION

The pesticides detected in the blood samples of volunteers selected for this study are classified as insecticides, generally used by the farm workers to control different kinds of pests to protect their crops. Thin layer chromatography is simple, sensitive and cost effective technique by which one can easily evaluate the pesticide burden in human blood samples. The presence of pesticide residues in volunteers (non-occupational) is also very alarming and indicative of environmental exposure spraying of pesticides in field. Chlorpyrifos, an organophosphorous insecticide detected in 40% of samples of male volunteers. After studying various other factors it is assumed that the presence of chlorpyrifos in blood sample is due to the massive use of this pesticide. The presence of deltamethrin residues in child blood sample (18 month) is also very alarming and indicative of environmental exposure. In conclusion, the results of this study may be useful in becoming a part of report for the contamination level of pesticide residues

in the blood samples of residents of Rajasthan with different socioeconomic characteristics, so that the sources and trends of this contamination may be identified.

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