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Title: **Pesticides contaminated dust exposure, risk diagnosis and exposure markers in occupational and residential settings of Lahore, Pakistan.**

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Highlights:

- ✓ This is the first assessment of pesticides using indoor dust, urine from Pakistan.
- ✓ Combined influence of various factors explains pesticides content in studied sites.
- ✓ Site specific differences were observed for Pesticide concentration.
- ✓ Significant variations were noted in pesticides biomarkers level in exposed group.
- ✓ Health status markers indicate that occupational groups at greater risk.

Abstract

There are few studies documenting the dust loaded with pesticides as a potential non-dietary exposure source for occupational worker and populations living near agricultural farms and pesticides formulation plants. In present study we have evaluated the pesticide concentration in dust from potential sites and relevant health risk from dust ingestion. Furthermore, the effect of currently used pesticides was investigated on blood and urine parameters of subjects: farmer, factory worker, urban resident and rural resident and controlled subjects with presumably different levels of exposure. The urinary metabolites (TCPY and IMPY) were quantified as biomarkers of exposure to chlorpyrifos and diazinon in relation with biomarkers of effect including BuChE, LH, FSH, testosterone and oxidative stress. Results showed that chlorpyrifos and diazinon were present in higher concentration in dust and posed a high health risk to exposed subjects. The mean SOD value was high among the farmer (3048 U/g Hb) followed by factory worker (1677.6U/g Hb). The urinary biomarkers - TCPY and IMPY- were found higher in exposed subjects as compared to control. Furthermore, testosterone was found in higher concentration in factory worker than control (12.63ng/ml vs 4.61ng/ml respectively). A decreased BuChE activity was noticed in occupational group and significant differences were observed in control verses exposed subjects. The PCA analysis evidenced the impact of pesticides on exposure biomarkers and male reproductive hormones. The study suggests that dust contaminated with pesticides engenders significant health risk particularly related to the nervous and endocrine system, not only for occupational workers exposed to direct ingestion but also for nearby residential community. Succinctly putting: Pesticides loaded dust in the city of Lahore, being a high priority concern for the government of Pakistan, demands to be addressed.

Keywords: pesticide, health markers, EDI, SOD, testosterone, BuChe, dust

1. Introduction

The potentially adverse effects of pesticides exposure on the general population are a public health concern (EFSA, 2013). At low levels, human beings are exposed to a variety of pesticides via contaminated food (Mercier et al., 2011) and household use (Trunnell et al., 2013). Nevertheless, the occupationally exposed subjects or populations living near pesticide formulation plants are exposed at far-off higher dose (Garcia, 1998). Variable working groups (e.g. farmers, horticulturists, pest controllers and formulation plant employees) are exposed to pesticides regularly. Several report ill health symptoms following exposure (Ross et al., 2010).

Indoor dust has been allied with human exposure to numerous organic contaminants and considered as a marker of indoor pollution (Bornehag et al., 2005) due to its significance as a sink and repository for particulate matter (Mannino and Orecchio, 2008). Monitoring and governing the occurrence of these contaminants in the surroundings is of great importance as they cause a risk to human and environmental health (Yusa etal., 2014).

The biochemical markers have been widely used in environmental monitoring studies to assess the impacts of single/mixture of pollutants (Yusa etal.,2015; Freitas et al., 2015; Velez et al., 2015). The toxicity of pesticides also appears to be related to their biotransformation into

responsive metabolites caused in reactive oxygen species (ROS) generation. The ROS (H_2O_2; O^-; OH) are capable of inducing lipo-protein oxidation, cell damage and depletion of endogenous antioxidants (Abdollahi et al., 2004). Some studies have used biomarkers to biologically monitor the uptake of individual pesticide compound from occupational exposures. A group of metabolites of pesticides -IMPY, TCPY- have been suggested as urinary biomarkers of human exposure to chlorpyrifos and diazinon respectively. The butyrylcholinesterase (BuChE) activity has also been used as biomarker of internal dose following recent contact to pesticides (Singh et al., 2007) Human exposure to organophosphate (OP) insecticides, carbamates and pyrethroids has been notably related with altered serum hormone concentrations and other endocrine disrupting effects (Meeker et al., 2008).

Pakistan, an agrarian society, is presently involved in consumption, formulation and import of pesticides and the apprehension is rising. Pakistan is the 2nd prime consumer of pesticides among the South Asian countries. Agriculture yield is basically reliant on the use of pesticides (Panhwar et al., 2014). Organophosphorus pesticide poisoning is a significant clinical problem in rural districts of the developing world, and exterminates an estimated 200, 000 people every year. Concerning fertilizers and pesticides, Pakistan has no manufacturing facility for pesticides. The native necessities are met by companies, which just formulate pesticides (Jaspal and Haider, 2014). The limited formulation products include liquid pesticides, powder and granules and contribute 67% in the local market. Most of the raw materials for preparation including active ingredients and pesticides in finished form are being imported *(PNP*, 2009*)*. Thus current use of pesticides is still on the upswing in Pakistan with a central use of pyrethroids followed by carbamates and OPs. (FAOSTAT, 2016). Agricultural Research Council (PARC) Pakistan apprises that approximately 10,000 cotton crop harvesting labors are yearly affected by

unmethodical use of pesticides (Illyas, 2012); (Nutkani, 2012). Likewise worldwide numerous studies have reported exposure of farm workers to pesticides. The levels of metabolites reported in their urine and blood are greater than the nonagricultural populations of same areas.(Rothlein et al., 2006; Shomar et al., 2014).

To date, there has been no to limited information on current use pesticides contaminants in Pakistan or an evaluation of correlations between dust intakes and concentrations of exposure markers in humans. Therefore, we carried out a study to evaluate indoor dust exposure in the occupational and residential settings exposed to non-persistent currently use pesticides, examining the relation between urinary biomarkers and the effect of pesticides exposure on oxidative stress, reproductive hormonal level and (BuChE) activity.

2. Material and methods

2.1 Study area description and selection

Lahore is located in northeast, semi-arid region lies between 31°35’25.4’’ N and 74°19’57.0’’ E and capital of Punjab province, Pakistan. The megacity had a population of 10.5 million, undergoing rapid population growth of four percent per year. The metropolitan city forms one of the most industrialized zone on Asian subcontinent, highly exposed to environmental problems associated with industry, agriculture and occupation. Indiscriminate land-use pattern showed penetrating industries in the agricultural and residential land. Similarly two major industrial estates, Quaid e Azam industrial estate and sunder industrial estate, Lahore were surrounded by urban residential and agricultural area respectively. In addition to industrial estates there were scattered industries throughout Lahore (Ghauri et al., 2007; Ali and Athar, 2010). A directory was generated after surveying and collecting all the information regarding pesticide industry and retailing stores as given in S1 table 7. Pesticides industries were mainly located along Rohi nalaa

; Sunder industrial estate; Quaid e azam industrial estate; kotlakhpat; katar bund road; Kacha jail road; Multan road; Ferozpur road and Thokar niaz baig (see figure 1).

2.2 Sampling strategy:

Indoor dust samples (n=50) were collected from pesticide formulation plants and pesticide distributor stores, urban and rural residential area. Each sample was a composite of three subsamples. Disposable pre cleaned Plastic brushes and dust pans were used for each time to collect the samples to avoid any pre contamination. All samples were wrapped in solvent rinsed aluminum foil to protect them from sunlight packed them in zipper bags already labeled for transport to the laboratory.

2.3 Subject selection and biometric parameters:

A brief interview was conducted to collect information on their work related parameters, health status and socio demographic status including age, height, weight, gender, working hours, work experience (see SI table 1). Adult male participants were enrolled; excluding subjects were those with extreme body weight and suffered from chronic diseases. An inclusion criterion of the subjects was based on occupational exposure or proximity to agricultural farm lands and pesticide manufacturing industries. The subjects meeting the criterion was selected from the total cohorts (n=502) comprising rural resident (n =121); urban resident (n=145); shopkeeper (n=25); farmer (n=150); factory worker (n= 61). The studied subjects were adult male with age ranging between (20 to55). The control subjects (n=50) were selected from the north of Pakistan comparatively less polluted with no industries and farm lands. All workers and residents were fully informed during on site interview about the details on research projects and expected outcomes. All subjects were recruited with written informed consent under an approved protocol

from the ethical board of the department of environmental science, Quaid e Azam University, Islamabad Pakistan.

2.4 Sod Assay:

SOD activity in human red blood cells was done using scheme described in detail (Winterbourn et al., 1975). Briefly, pyrogallol was used as a substrate agent and final absorbance was taken at 420 nm.

2.5 BuChE assay:

Content of BuChE activity was determined using Kit (Doles Reagent) within 24 hours after blood collection. DTNB was used that produces a yellow color upon contact with thiocoline. Final absorbance was measured at 405nm by spectrophotometer.

2.6 Endocrine Disruption:

Levels of LH & FSH were determined by using sequential-chemi-luminescent immune-metric assay in an automated mode analyzer (Immulite 1000) Germany. The testosterone content was calculated using radiometric- immunoassay in an automated mode analyzer. (Bio-line) Belgium.

2.7 Pesticide metabolite detection in urine:

Two metabolites of organophosphate pesticides, Chlorpyrifos and diazinon (TCPY and IMPY) respectively were analyzed. Quantification of metabolites was done by HPLC- MS. The method was described in detail in (Sams C., 2007). Shortly, 2 ml of urine sample was spiked with internal std. then sodium hydroxide (0.5 Molar) was used to alkalize the reaction mixture. Liquid-liquid extraction method was used with cartridges -Chemi elute- adding formic acid and

ethyl acetate. The elute solvent was dried under gentle stream of nitrogen then reconstituted with methanol.

2.8 Gas chromatography-Mass spectrometry Analysis

2.8.1 Reagents and Standards

Ethyl Acetate, hexane and acetone were HPLC gradient solvents from Fisher Scientific UK. Sodium sulphate (Na_2SO_4) was obtained from Sigma Aldrich and alumina was obtained from Merck in Germany. The recovery standard labeled d-10 chlorpyrifos diethyl, toclofos, diazinon, pirimicarb, cyprodinil, azinphos; deltamethrine was obtained from Cambridge Isotope Laboratories Inc. The stock solution of the recovery standard was 100 ug/ml in nonane and was diluted to 1 ug/ml in hexane. Analytical grade triphenyl phosphate (TPP-d15) was purchased from QMX Laboratories Ltd, Thaxted, UK. TPP was used as an internal standard. A 100 ug/ml TPP was prepared using acetone as the solvent. A stock solution of 1000 ug/ml chlorpyrifos was prepared in acetone using analytical grade chlorpyrifos-ethyl obtained from Sigma-Aldrich UK. From mix stock solution seven calibration curves were prepared and solvent exchanged to hexane. A blank containing hexane was included.

2.8.2 Analytical setup

The samples were analyzed using a Finnigan TRACE GC-MS system, consisting of a Phenomenex ZB-Multiresidue-2 GC column (30m×0.25mm×0.2um). The initial oven temperature was 70 °C (held for 2min), then increased to 150 °C at a rate of 25 °Cmin^{-1}, further increased to 220 °C (3°C min^{-1}), and finally to 300 °C (10 °C min^{-1}) where it was held for 10 minutes. The GC interface temperature was set to 300 °C and the MS source temperature to 250 °C.

2.8.3 Sample preparation

The dust samples stored in freezer were allowed to thaw. About 1 g of each sample was transferred into a centrifuge tube. The dust sample in the centrifuge tube was dried using 3 g baked anhydrous Na_2SO_4. The dust Na_2SO_4 mixture was thoroughly mixed into a fine powder. The dried sample was spiked with 50ug of 1ug/ml d-10 chlorpyrifos in hexane as a recovery standard. A blank containing 3 g of Na_2SO_4 was included after every 10 samples.

2.8.4 Sample extraction and clean up

The samples were extracted with 30 ml 2:3 Hexane: Ethyl acetate mixture. 10 ml of the extracting mixture was added to spike Na_2SO_4 dried samples in centrifuge tube, hand-shake for 10 minutes and centrifuged at 2000 rpm for 2 minutes. The extract was decanted and the extraction was repeated three times. The volume of the extracts was reduced to about 1ml using a slow stream of nitrogen gas at a temperature of 40 °C. The extract was cleaned using solid phase chromatography glass column in which 6 g of alumina and 1 cm thick sodium sulphate were added. The column was rinsed with 20 ml ethyl acetate before adding the samples. The extract was eluted through the column with 20 ml ethyl acetate. The volume was reduced to about 0.5ml using slow stream of nitrogen gas and then transferred to a 2 ml vial to which 10 ul of 100 ug/ml TPP was added. The final solution was blow dried and dissolved with 1ml Hexane. The samples were analyzed using a Finnigan Trace GC-MS.

2.9 Potential Health risk to population via dust ingestion:

The estimated daily intake (EDI) for pesticide compounds are given in equation1.

$$EDI = \frac{CD \times DI \times FS}{BW} \quad \text{……Equation 1.}$$

The above equation revealed estimated daily intake of pesticides loaded with dust. Where CD (ug/g) represent the concentration of individual pesticide compound in dust, DI indicate daily

intake of dust g/day, FS is the fraction of time spent at workplace or home, BW represent the body weight of studied subjects.

The hazard quotient (HQ) is determined by equation 2 given as.

HQ = EDI/RfD…….EQ-2.

In the above equation EDI represent estimated daily intake and (RfD) is reference dose estimated by EPA for different pesticides. If the value of HQ is greater than that means exposed subjects/ population is at risk with adverse health consequences.

2.10 Statistical analysis:

Descriptive statistical parameters including range, mean, St. deviation and median were calculated with help of SPPS version 21. Concentration bar graphs and error bars were devised by means of Microsoft Excel 2007. ANOVA and Correlation on the basis of variables were calculated with SPSS 21. For Classification of sites principal Component analysis ordination technique was applied and PCA biplots were plotted with MVSP 3.2. Regression analysis and line graphs were also devised via Microsoft Excel 2007.

3. Results and discussion

The demographic features along with occupation based information including working hours and work experience of the subjects (Farmer, factory worker, shopkeeper, rural resident, and urban resident) were presented in SI table 1. Pesticide factory worker worked an average of 8 h/day while a farmer 10 hours daily. No significant differences were recorded between age, height and weight of studied subjects.

3.1. Dust pesticide profile

The concentration profile of seven pesticide compounds in the dust matrix collected from different landuse was shown in table 1. Among the investigated analytes, the chlorpyrifos was the predominant compound with concentration ranging from 4 - 15.1 ug/g. Furthermore, distribution trend of studied compounds were, industrial area > rural residential area > urban residential area > pesticide distribution stores. The organophosphate pesticides were the prominent compounds (chlorpyrifos and diazinon) followed by deltamethrine > pirimicarb > cyprodinil > azinphos > toclofos. One way anova indicated that significant differences exist among pesticides analyzed under specified zones. Regarding indoor pollutants, settled dust has been considered as essential contact medium. Dust pesticides data are scarcely available for Pakistan, what makes it hard to contextualize our data for comparison purposes. However, the dust collected from houses or near farm settlements reveled that our residential and rural pesticide profile except chlorpyrifos is comparable to the reported studies. Trunnell etal., 2013; Rubino etal., 2012; Ostrea etal., 2012 ; Chensheng etal., 2004.

3.2. Urinary metabolites of pesticide

Urinary metabolites were estimated for Chlorpyrifos and diazinon from matched subjects from where dust samples were collected as shown in table 2 shows the distribution pattern of urinary biomarkers among subjects. The comparative analysis among susceptible subjects showed a decreasing trend of urinary level of OP metabolites (TCPY, IMPY) i.e. factory worker > farmer > shopkeeper > urban and rural resident. Comparatively, the insecticide metabolite was much higher in factory worker than that of control (95th percentile 2.1 and 2.3 ng/ml) vs. 41.1 and 10.5 ng/ml in control and factory worker respectively), followed by farmer 37.9 and 12.9 ng/ml for TCPY and IMPY respectively. In exposed humans, a significant proportion of OP insecticide is absorbed by all three routes of exposure i.e. inhalation, ingestion and very significant dermal

routes of absorption). The high levels of urinary metabolites (TCPY, IMPY) in rural and urban resident showed that in the city of Lahore, dust dispersing from industrial and agricultural farms were major contributors in insecticide exposure in addition to food. These findings are evident to the high urinary concentration of TCPY and IMPY in exposed population compared with previously reported literature from other parts of the world (Table 1). Specific OP insecticide's metabolite of chlorpyrifos and diazinon (TCPY, IMPY) were studied in various bio-monitoring studies mostly in children and general population to measure the exposure extent of specific pesticide (CDC, 2012; Panuwet et al., 2009 ; Castorina et al., 2010). The most repeatedly determined metabolite is TCPY representing chlorpyrifos dose, which is present in about 75% of the samples, with mean concentrations in the series of 1-3 ng mL^{-1}(EU, 2014).

3.3 Biomarkers of pesticide exposure and oxidative stress

The health risk associated with exposure to insecticides is the consequence of disturbance in the antioxidant defense system, resulting in oxidative stress (Fatma etal., 2013; Kale etal., 1999; Azaroff,1999)). Large amount of reactive oxygen species are generated during interaction with pesticides which disturb the cell homeostasis (Karami- Mohajeri and Abdollahi, 2011). Comparative analysis of the average activity of SOD in the exposed vs. control is shown in figure 2. The mean SOD value was high among the farmer (3048 U/g Hb) followed by factory worker (1677.6U/g Hb) > shopkeeper (1558 U/g Hb) > urban resident (1537 U/g Hb) > rural resident (1372 U/g Hb). Overall, the SOD values were higher than the control subjects 1237.6 U/g Hb. However, no significant differences were noted among subjects (see fig 2). High antioxidant (SOD) activity in exposed subjects showed that they suffer from oxidative stress and possibly related to the enhanced H_2O_2 production, because pesticides are known for their

potential to cause oxidative stress by releasing free radicals (Soltaninejad and Abdollahi, 2009; Abdollahi et al., 2004; De Silva et al., 2006; Shadnia et al., 2005)

3.4 Butyrylcholinesterase (BuChE)

The cholinesterase originates in blood serum and produced by liver has been labeled pseudo- or butyrylcholinesterase (BuChE). Blood cholinesterases are widely used for observing acquaintance to organophosphorus and carbamate pesticides. There are strong associations between exposures to pesticides and symptoms. Moreover, cholinesterase is significantly reduced in exposed populations (Nigg and Knaak, 2000; Mourad, 2005; Singh et al., 2007; Ali et al., 2008). For this purpose the amount of blood BuChE activity has been reflected a good biomarker for this kind of exposure. The mean BuChE activity recorded in the subjects were in the descending order; urban resident (3.76)> rural resident (3.56) > farmer (3.48) > shopkeeper (3.35) > factory worker (2.77) U/ml. Moreover, there was significant differences observed in BuChE activity in control verses exposed subjects. (P = > 0.05) (See figure 2). The BuChE is a key enzyme for the nervous system and is accountable for the degradation of the acetylcholine. If acetylcholine rests in neural synapses, it can interrupt the regular working of the nervous system. Organophosphates can disturb cholinesterase action and consequence in the accretion of acetylcholine in synapses (Panemangalore et al., 1999; Ali et al., 2008). The substantial decrease in the concentration of BuChE in exposed workers designates that contact to pesticides are likely to disrupt nervous system function. The exposure of the insecticides, by farm, factory workers and exposed population in this study, could justify these findings. These findings are consistent with those of former studies (Hernández et al., 2005; Mourad, 2005; Ali et al., 2008), and ratify that the amount of plasmatic cholinesterase can be valuable as biomarker in the monitoring of inhabitants exposed to organophosphorus and carbamates pesticides.

3.5 Endocrine disruptors

The use of hormonal quantification is very useful in the world of exposure assessment when dealing with the decreased fertility rate in certain occupations (Ashiru and Odusanya, 2009). Male reproductive health has deteriorated during the last few decades. Pesticides have been demonstrated as EDs that contribute to male infertility. They are able to modify serum level of hormones in exposed human by interfering with the normal regulation processes. In this study we have assessed the exposure of five groups, which are valuable classes to pesticide exposures. The mean FSH level reported in studied groups were farmer (1.12 mlU/ml) > factory worker (0.70 mlU/ml) > rural resident (0.63 mlU/ml) > shopkeeper (0.48 mlU/ml) > urban resident > (0.47 mlU/ml). Overall, the values were higher than control (0.41 mlU/ml) as shown in figure 2.

The LH level calculated in exposed subjects revealed a pattern as followed; farmer (12.78 mlU/ml) > shopkeeper (10.67 mlU/ml) > rural resident (10.41 mlU/ml) > factory worker (8.34 mlU/ml) > urban resident (6.14 mlU/ml) as given in figure 2.

Testosterone plays a major role in the instruction of germ cell growth and the preservation of male fertility. The testosterone level recorded were in descending order as farmer (12.63ng/ml) > factory worker (11.69ng/ml) > rural resident (11.25 ng/ml) > urban resident (6.15ng/ml) > shopkeeper > (5.11ng/ml). However, the measured testosterones in exposed subjects were higher than control (4.61ng/ml). Testosterone is formed by the leydig cells in retort to stimulation with LH and acts as a paracrine element that diffuses into the seminiferous tubules. (Holdcraft and Braun., 2004; Li H.J., 2014; Smith and Walker., 2014)

It has been designated that human exposure to OP insecticides, carbamates and pyrethroids notably related with altered serum hormone concentrations and other endocrine disrupting effects

(Meeker et al., 2008). Several Biomonitoring studies regarding occupationally exposed subjects and general population, report alterations in the normal level of reproductive hormones. Miranda-Contreras etal.,2013 ; Aguilar-Gardu etal., 2013; Meeker et al., 2006; Blanco-Mu etal.,2010; Recio etal.,2005; Padungtod etal., 1998

3.6 Correlation and regression analysis:

The studied pesticide compounds had a significant and positive correlation with FSH, LH, testosterone and SOD as given in SI table8. Significant negative correlations were observed for pesticides (diazinon, pirimicarb, chlorpyrifos deltamethrine) with BuChE.

The regression coefficient (see figure 4) revealed that 74% and 53% of the activity of testosterone and FSH respectively was accounted for by Σ pesticides exposure which shows that pesticides were mainly responsible for enhanced testosterone activity. There might be other factors involved in that phenomenon. However, pesticides played a significant role in male reproductive hormonal induction and signify as endocrine disruptors.

3.7 Principle component analysis:

The principal component analysis showed two principal factors accounting for 75% of the variability of the data set. The two components represented 34% (PC1), 25.1% (PC-2) of the variability respectively and were represented in Euclidean biplot (see Fig. 3). A Euclidean biplot is the plot of normalized eigenvectors. The length of the eigenvector indicates the contribution of the variable to ordination space. Furthermore, an eigenvector approaching a length of 1 indicates that a variable contributes strongly to defining the ordination space. In the Euclidean biplot, exposed and control groups were distinctly clustered in different quadrants. The first factor comprised of cyprodinil, azinphos, toclofos, diazinon, pirimicarb, chlorpyrifos and FSH, LH,

testosterone. The Euclidean biplot showed aggregate of exposed group around this component. The cluster of farm worker was more closely associated with testosterone, diazinon, pirimicarb and LH. However, other exposed groups (shopkeeper, pesticide factory worker, rural residents) were more scattered around LH, FSH, testosterone and determined pesticide compounds. The exposed group showed closed association with the pesticides and hormonal levels; on the other hand control group was more concentrated away and clustered around the second factor with BuChE and SOD. Overall, PCA showed that exposed groups (farmer, shopkeeper, factory worker) were more exposed to pesticides and resulting enhanced reproductive hormonal levels as compared to control. However, a decreased antioxidant activity (SOD) and BuChE level in the exposed subjects signify their grouping away with the control group.

3.8 Health risk via dust inhalation

The cumulative health risk of pesticide ingestion via dust residues was presented in SI Table 1. Evaluated results revealed remarkably high risk of exposure for chlorpyrifos and diazinon by dust media; HQ exceed 1 (Threshold value) for all the exposed groups. However, the occupational group (pesticide factory worker and store keeper) revealed a relatively high hazard as the value is far higher than 1 for chlorpyrifos and diazinon. The magnitude of risk from each target contaminant ranked in order of Chlorpyrifos > Diazinon > azinphos > Deltamethrine > Cyprodinil > toclofos. The highest estimated daily intake was cumulated for Chlorpyrifos (0.07) ug kg^{-1} bw d^{-1} in comparison to rest of pesticides. Hazard Quotient (HQ) for each contaminant was quantified with the RfD values specified US-EPA. Since non- carcinogenic Chronic Oral Exposure doses have not been evaluated for some pesticides there risk could not be calculated. The RfD values in terms of chronic exposure and US EPA recommended exposure factors are listed in SI table 4.

The results reported in this study supporting studies highlighting ingestion as a core route of exposure in terms of pesticides. However, literature majorly focuses on pesticide ingestion via food instead of dust. Secondly inhalation by dust bound pesticide is more pronounced as compared to soil bound residues. Study performed by (Simcox et al., 1995) reported greater sorption of Azinphos to dust particles in relation to soil media, making dust the prominent exposure medium. While model based assessments revealed that non-dietary uptake of chlorpyrifos was dependent on amount of surface bound residues (Zartarian et al., 2000). Nonetheless our estimates were coherent with the findings of (Wenrui et al., 2009). Several studies had been done on bio monitoring of organophosphate pesticides in urine, blood etc. of farm workers and agricultural families living nearby farms (Anwar, 1997; Azaroff, 1999; Azmi et al., 2006; Lu et al., 2000; Mills and Zahm, 2001; Panuwet et al., 2008). According to (Ross et al., 2010) , there is no biomarker of long term exposure to very small concentrations of organophosphate pesticides. Estimated HQ predicts risk to residential urban and rural population potentially exposed to multiple organophosphate and pyrethroids pesticides. However current-use pesticides are typically formulated with synergists (Saillenfait et al., 2015) Thus, cumulative risk should not be ignored.

Conclusion:

Current study presents pesticide exposure among subjects exposed to pesticides contaminated dust in Lahore city, Pakistan. The results indicated that the pesticide loaded dust pollution is a health risk factor for factory worker, farmer, and surrounding residential population. In addition to this, all exposed subjects experience oxidative stress, endocrine disruption and nervous stress. The high urinary level of TCPY and IMPY indicates that the chlorpyrifos and diazinon

formulation and over use in agricultural farms is a serious health risk and needs to be addressed on priority basis for worker and general population health safety in Pakistan.

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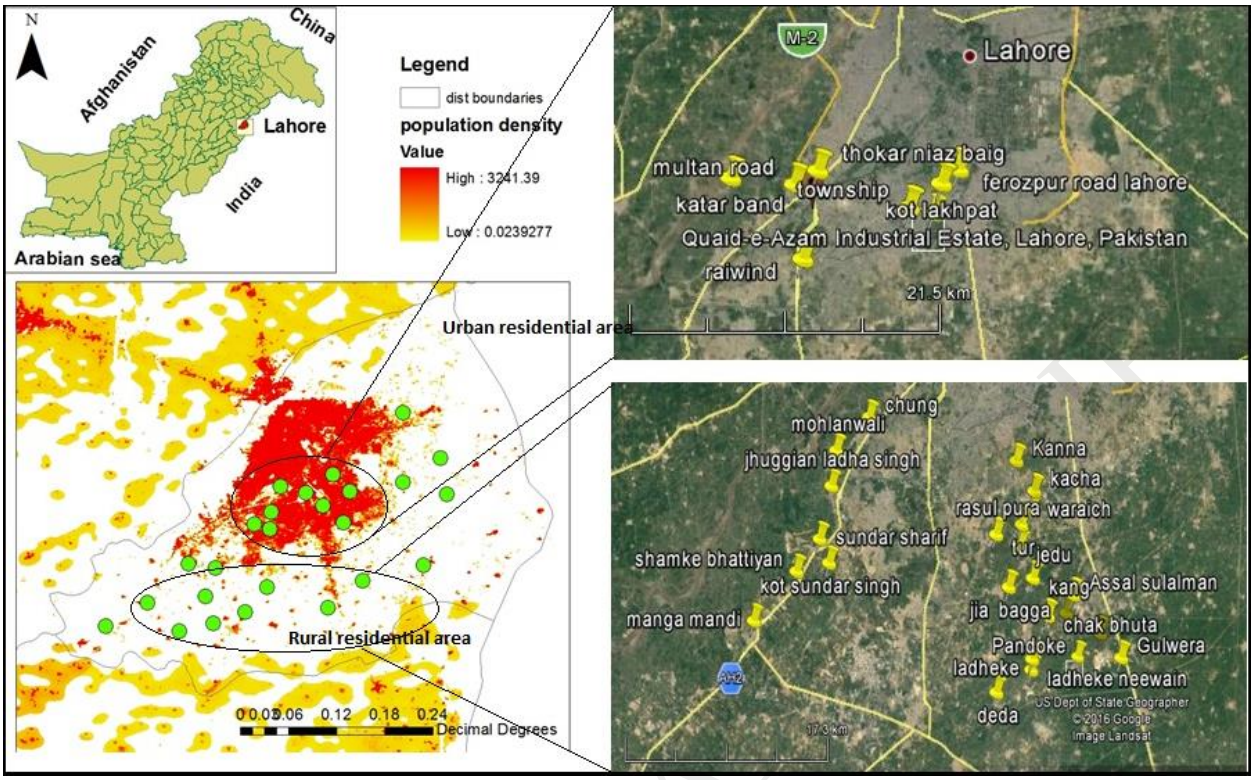


Figure 1 Sampling sites, Lahore Pakistan

Table 1 Pesticide profile of dust from potential land-use, Lahore

ug/g		Diazinon	Pirimicarb	Toclofos	Chlorpyrifos	Cyprodinil	Azinphos	Deltamethrine	p value
Rural residential area	Mean	0.203	0.026	0.007	7.846	0.037	0.014	0.014	<0.05
	St.D	0.049	0.001	0.004	0.606	0.042	0.009	0.009	
	Min	0.040	0.024	0.002	6.068	0.002	0.002	0.002	
	Max	0.260	0.028	0.012	8.248	0.156	0.024	0.024	
Urban residential area	Mean	0.246	0.026	0.012	7.369	0.072	0.026	0.023	<0.01
	St.D	0.010	0.002	0.000	1.327	0.039	0.000	0.003	
	Min	0.240	0.023	0.012	4.000	0.032	0.026	0.020	
	Max	0.260	0.028	0.012	8.416	0.122	0.026	0.028	
Pesticide distributor stores	Mean	0.104	0.031	0.009	6.279	0.024	0.024	0.020	0.03
	St.D	0.058	0.004	0.003	0.098	0.009	0.003	0.002	
	Min	0.016	0.027	0.002	6.118	0.014	0.016	0.016	
	Max	0.180	0.039	0.012	6.422	0.038	0.026	0.024	
Industrial area	Mean	0.695	0.072	0.010	10.017	0.058	0.021	1.664	<0.05
	St.D	0.370	0.056	0.003	2.341	0.039	0.008	1.507	
	Min	0.200	0.020	0.004	6.756	0.002	0.004	0.026	
	Max	1.260	0.180	0.012	15.040	0.120	0.026	3.660	

One way anova indicate pesticides compound significantly vary (p=>0.05) among selected land-use groups

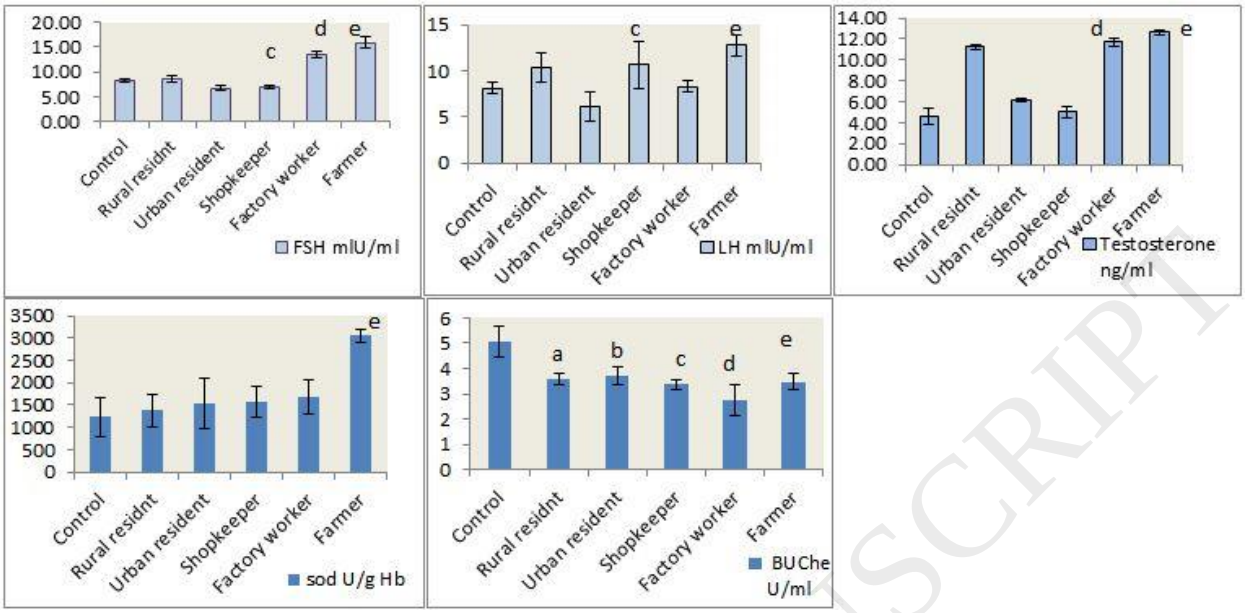


Figure 2 Comparative analysis of blood biomarkers of effect in exposed verses control subjects

a = control verses rural resident

b = control verses urban resident

c = control verses shopkeeper (pesticide store keeper)

d = control verses factory worker

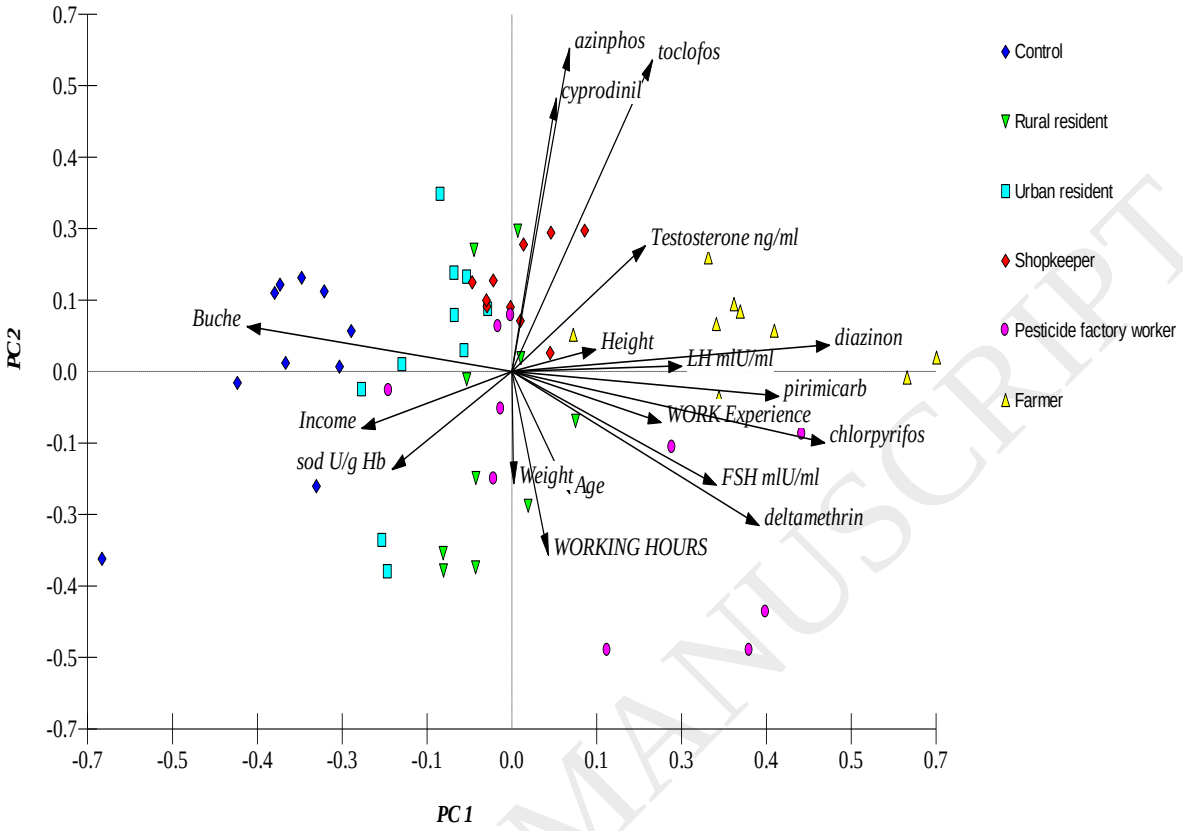
e = control verses farmer

Table 2 Comparative analysis of urinary biomarkers of exposure to OP pesticides

Pesticide	Metabolite	ng/ml	N	Percentiles					
				LOD	GM	50th	75th	95th	Max
Chlorpyrifos	TCPY	Control	50	0.1	0.37	0.4	0.9	2.1	3
		Rural resident	121	0.1	0.8	1.1	2.1	4.2	26
		urban resident	145	0.1	0.39	1	0.5	1.7	12
		shopkeeper	25	0.1	0.4	1	1.5	3.8	17
		farmer	150	0.1	1.9	1.1	10.5	37.9	198
		factory worker	61	0.1	2.29	1.95	4.81	41.1	258
Diazinon	IMPY	Control	50	0.1	0.2	<LOD	0.7	2.3	4.4
		Rural resident	121	0.1	0.81	0.47	1.7	2.7	5.2
		urban resident	145	0.1	0.1	0.5	1.7	2.9	5.6
		shopkeeper	25	0.1	0.11	0.8	2.9	4.5	29
		farmer	150	0.1	0.7	0.3	1.21	12.9	76
		factory worker	61	0.1	0.91	0.5	2.3	10.5	160

TCPY= 3, 5, 6, trichloro 2 pyridinol

IMPY= 2 isopropyl 4 methyl 6 hydroxy pyrimidine



Vector scaling: 1.19

Figure 3. Principal component analysis of biological parameters verses dust pesticide concentration in exposed and control subjects.

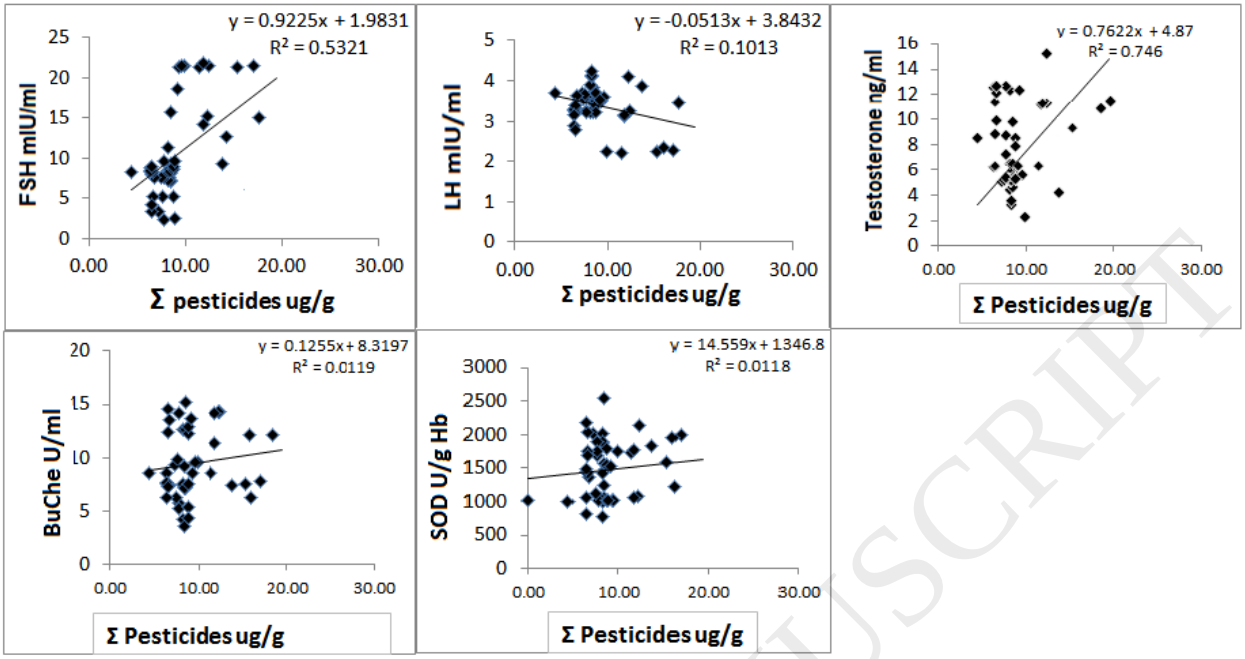


Figure 4 Regression plots of detected pesticide Σ concentration in dust matrix and biological parameters.

LH= luteinizing hormone

FSH= follicle stimulating hormone

SOD= superoxide dismutase

BuChe = Butyrylcholinesterase