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Monitoring of Pesticide Residue Levels in Blood Samples Collected from the Agricultural Spray-workers in Menoufia Governorate, Egypt

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ABSTRACT

Spray-workers during the activity of spraying crops are directly exposed to pesticides while mixing handling and spraying. Also, they are exposed to pesticides indirectly through contaminated soil, air, drinking water, eating food, and smoking at workplaces. Thus, the aim of this study was to monitor pesticide residues in blood samples collected from exposed agricultural workers (spraying workers) from three different locations (Shibin El-Kom, Tala and Quweisna) in Menoufia Governorate, Egypt during 2021. A total of 461 Pesticides were analyzed using HPLC and GC-MS/MS system to determine pesticides residues in blood samples of spray workers. Ten pesticides, one herbicide (atrazine), four insecticides (chlorpyrifos, cyhalothrin-lambda, DDE-p,p' and malathion), four fungicides (metalaxyl, ortho phenylphenol, propiconazol and pyrimethanil) and one pesticide synergist (piperonyl butoxide) were detected. Results shown that the most frequently pesticide found in blood samples of spray workers was chlorpyrifos (in 7 blood samples) with percent 35% followed by malathion (in 4 blood samples) with percent 20%. The highest concentration of pesticide residue in analyzed spray workers blood samples was pyrimethanil fungicide with 0.035 mg/kg in blood samples from Quweisna.

Keywords: Spray workers, Pesticide residue, Blood samples, QuEChERS technique.

INTRODUCTION

Pesticides are used to improve agricultural production and control pests, but there is a possibility that they will have unfavorable effects on the environment and human health. During the process of spraying crops, spray-workers are directly exposed to pesticides when handling, mixing, and spraying. Also, they are exposed to pesticides indirectly through contaminated soil, air, drinking water, eating food, and smoking at workplaces. Finally, these are absorbed by inhalation, ingestion, and dermal contact (Vega, 1994).

Agricultural workers in developing countries are exposed to pesticide contamination (occupational exposure) due to improper application techniques, poor or unsuitable spraying equipment, insufficient storage practices, a lack of personal protective equipment, and the reuse of old pesticide packaging for food or water storage (Ecobichon, 2001; Damalas and Eleftherohorinos, 2011; Nassar et al., 2016). Furthermore, pesticides were applied to crops by non-certified applicators (farmers) using handled, back-held, or motor-drawn sprayers, putting farmers at risk of exposure.

Pesticide exposure has been linked to a variety of negative health effects, including acute symptoms such as abdominal pain, dizziness, headaches, nausea, vomiting, skin problems, eye irritation, and excessive salivation, as well as chronic diseases such as cancer, reproductive, and developmental disorders. Restlessness, memory loss, convulsions, and coma are all frequent effects on the Central Nervous System (CNS). Furthermore, there have been several reports of effects on the parasympathetic and sympathetic nervous systems, including respiratory paralysis, which is lethal (US EPA, 2005).

During 2016, Egypt consumed 10,600 metric tons of pesticides (active ingredient), accounting for 0.2 percent of global usage (Sustainability of Agricultural Environment in Egypt: Part II). According to the Agricultural Pesticides Committee, (2019) Egypt used about 11,000 tons (active ingredients) of pesticides in 2018 (4,510; 4,290; and 2,200 t of herbicides, insecticides, and fungicides, respectively).

Therefore, the objective of this study was to monitor pesticide residues in blood samples collected from exposed agricultural workers (spraying workers) living in Menoufia Governorate, Egypt during 2021.

MATERIALS AND METHODS

1. Study area

This study was carried out on pesticide spraying workers living Menoufia governorate, Egypt, during 2021. Menoufia is governorate of Lower Egypt in the western part of the apex of the Nile River delta, between the Damietta (east) and Rosetta (west) branches of the Nile. Agriculture is the principal occupation, employing more than three-fifths of the workers. Nearly one-third of the land is under corn (maize); other crops include cotton, wheat, clover, dates, and onions (Britannica, 2012).

2. Collection of blood samples:

Blood samples were collected from field workers involved in pesticides spraying activities at three different locations (Shibin El-Kom, Tala and Quweisna) in Menoufia Governorate. For this issue, blood samples of 30 adult male ranging from 16-50 years old. Subjects were classified into 2 groups of people: Group 1 (spray workers, occupationally exposed) was of 20 spray workers exposed to pesticides continuously for about 5-10 years. Group 2 (control, not exposed to pesticides) was of 10 Inhabitants from the city who were not involved in the pesticide application.

For pesticide residues analysis about 5 mL of blood samples were collected with the help of a professional nurse into residue free heparinized glass vials with sterilized syringe after cleaning the skin with a swab of methylated spirit to minimize sample contamination from possible Pesticides residue adsorbed to the skin Blood samples were transported in dry ice to the laboratory and stored at -20˚C until they were analyzed.

3. Chemicals and pesticides used:

Organic solvents used such as methanol, acetonitrile, acetone (HPLC grade) and toluene (≥ 99.9%) were purchased from Merck (Germany). N-hexane, 97% was obtained from Sigma Aldrich or similar quality. De-ionized Water, generated by Millipore water-purification system was used. QuEChERS extraction reagents such as magnesium sulphate, sodium chloride, sodium citrate and citric acid disodium salt were purchased ready mixed in one package (Agilent Technologies). Pesticide reference standards (for about 461 pesticides Table 1.) obtained from Dr. Ehrenstorfer (Augsburg, Germany), with purities >95% were used to prepare stock solutions dissolved in Toluene for stock solution. Reference standard solutions of concentration 1000 µg/ml were prepared and were kept at -20±2˚C; for HPLC and GC-MS/MS using (Hexane: Acetone 9:1). The solvent(s) used are appropriate to the analyte solubility, stability and method of analysis (i.e., not negatively influencing on the pesticides).

Table 1. A total of 461 Pesticides of different groups analyzed using HPLC and GC-MS/MS system to determine pesticides residue levels in blood samples of agricultural spray-workers

No.	Compound	LOQ* (mg/kg)	No.	Compound	LOQ (mg/kg)	No.	Compound	LOQ (mg/kg)
1	1-NaphthyIacetic acid	0.05	**156**	Endosulfan-sulfate	0.01	**311**	Oxadiaxyl	0.05
2	2-(1-Naphthyl) acetamide	0.01	**157**	Endrin	0.05	**312**	Oxadiazon	0.01
3	Abameciin	0.01	**158**	EPN	0.01	**313**	Oxamyl	0.01
4	Acephate	0.01	**159**	Epoxiconazole	0.01	**314**	Oxasulfuron	0.01
5	Acetamiprid	0.01	**160**	Ethiofencarb	0.01	**315**	Oxycarboxin	0.01
6	Acrinathrin	0.01	**161**	Ethiofencarb Sulfone	0.01	**316**	Oxydemeton-methyl	0.01
7	Alachlor	0.01	**162**	Ethiofencarb Sulfoxide	0.01	**317**	Oxyfluorfen	0.05
8	Aldicarb	0.01	**163**	Ethion	0.01	**318**	Paclobutrazole	0.01
9	Aldicarb Sulfone	0.01	**164**	Ethirimol	0.01	**319**	Paraoxon-ethyl	0.01
10	Aldicarb Sulfoxide	0.01	**165**	Ethofumesate	0.01	**320**	Paraoxon -methyl	0.01
11	Ametoctradin	0.01	**166**	Ethoprophos	0.01	**321**	Parathion-ethyl	0.01
12	Ametryn	0.01	**167**	Ethoxyquin	0.01	**322**	Parathion-methyl	0.01
13	Amidosulfuron	0.01	**168**	Etofenprox	0.01	**323**	PCB 101	0.01
14	Arninocarb	0.01	**169**	Etoxazole	0.01	**324**	PCB 118	0.01

Table 1. (continued)

15	Amisulbrom	0.01	**170**	Etridiazole	0.01	**325**	PCB 138	0.01
16	Arnitraz	0.05	**171**	Etrimfos	0.01	**326**	PCB 153	0.01
17	Anilofos	0.01	**172**	Famoxadone	0.01	**327**	PCB 180	0.01
18	Atraton	0.05	**173**	fenamidone	0.01	**328**	PCB 28	0.01
19	Atrazine	0.01	**174**	Fenamiphos	0.01	**329**	PCB 52	0.01
20	Azaconazol	0.01	**175**	Fenamiphos sulfone	0.01	**330**	Penconazole	0.01
21	Azamethiphos	0.01	**176**	Fenamiphos sulfoxide	0.01	**331**	Pencycuron	0.01
22	Azimsulsfuron	0.01	**177**	Fenarimol	0.01	**332**	Pendimethalin	0.01
23	Azinphos-ethyl	0.01	**178**	Fenazaquin	0.05	**333**	Penoxsulam	0.01
24	Azinphos-methyl	0.01	**179**	Fenbuconazole	0.01	**334**	Pentachloroamisole (PCA)	0.01
25	Azoxysttrobin	0.01	**180**	Fenfurarm	0.01	**335**	Permethrin	0.01
26	Barban	0.01	**181**	Fenhexamid	0.01	**336**	Phenmedipham	0.01
27	Beflubutamid	0.01	**182**	Fenitrothion	0.05	**337**	Phenthoate	0.01
28	Benalaxyl	0.01	**183**	Fenoxaprop-p-ethyl	0.01	**338**	Phorate	0.01
29	Bendiocarb	0.01	**184**	Fenoxycarb	0.01	**339**	Phorate sulfone	0.01
30	Benomyl (as Carbendazim)	0.01	**185**	Fenpropathrin	0.01	**340**	Phorate sulfoxide	0.01
31	Bensulfuron-methyl	0.01	**186**	Fenpropidin	0.01	**341**	Phosalone	0.01
32	Benthiavalicarb isopropvl	0.01	**187**	Fenpmpimorph	0.01	**342**	Phosmet	0.01
33	Benzoximate	0.01	**188**	Fenpyrazamine	0.01	**343**	Phosphamidon	0.01
34	Bifenazate	0,01	**189**	Fenpyroximate	0.01	**344**	Phoxim	0.01
35	Bifenthrin	0.01	**190**	Fenthion	0.01	**345**	Picolinafen	0.01
36	Biphenyl	0.01	**191**	Fenthion sulfoxide	0.01	**346**	Picoxystrobin	0.01
37	Bispyribac-Sodium	0.01	**192**	Fenvalerate	0.05	**347**	Pinoxaden	0.01
38	Bitertanol	0.01	**193**	Fipronil	0.01	**348**	Piperonyl butoxide	0.01
39	Bixafen	0.01	**194**	Flamprop	0.01	**349**	Pirimicarb	0.01
40	Boscalid	0.01	**195**	Flonicamid	0.01	**350**	Pirimicarb desmethyl	0.01
41	Brodifacoum	0.01	**196**	Florasulam	0.01	**351**	Pirimiphos-ethyl	0.01
42	Bromacil	0.01	**197**	Fluazifop-p-butyl	0.01	**352**	Pirimiphos-methyl	0.01
43	Bromophos-ethyl	0.01	**198**	Flubendiamide	0.05	**353**	Prochloraz	0.01
44	Bromophos-methyl	0.01	**199**	Flucarbazone Sodium	0.01	**354**	Procymidone	0.01
45	Biomopropylate	0.01	**200**	Flucythrinate	0.01	**355**	Ptodiamine	0.01
46	Bromoxynil-octanate	0.01	**201**	Fludioxonil	0.01	**356**	Profenofos	0.01
47	Bromuconazole	0.01	**202**	Flufenacet	0.01	**357**	Profluralin	0.05
48	Bupirimate	0.01	**203**	Flufenoxuron	0.01	**358**	Profoxydim	0.01
49	Buprofezin	0.01	**204**	Flumetsulan	0.01	**359**	Promecarb	0.01
50	Butachlor	0.01	**205**	Flumeturon	0.01	**360**	Prometon	0.01
51	Butocarboxim	0.01	**206**	Fluopicolide	0.01	**361**	Prometryn	0.01
52	Butocarboxim subfoxide	0.01	**207**	Fluopyram	0.01	**362**	Propachlor	0.01
53	Butralin	0.01	**208**	Fluquinconazole	0.01	**363**	Propamocarb	0.01

Table 1. (continued)

54	Butylate	0.01	**209**	Fluroxypyr	0.01	**364**	Propanil	0.01
55	Cadusafos	0.01	**210**	Fluroxypyr-methyl	0.01	**365**	Propaquizafop	0.01
56	Captan	0.01	**211**	Flusilazole	0.01	**366**	Propargite	0.01
57	Carbaryl	0.01	**212**	Flutolanil	0.01	**367**	Propazine	0.01
58	Carbendazim	0.01	**213**	Flutriafol	0.0I	**368**	Propazine-2-hydroxy	0.01
59	Carbetamide	0.01	**214**	Fluxapyroxad	0.01	**369**	Propetamphos	0.01
60	Carbofuran	0.01	**215**	Foramsulfuron	0.01	**370**	Propiconazol	0.01
61	Carbofuran-3-hydroxy	0.01	**216**	Formetanate	0.01	**371**	Propoxur	0.01
62	Carbosulfan	0.05	**217**	Formothion	0.01	**372**	Propyzamide	0.01
63	Carboxin	0.01	**218**	Fosthiazate	0.01	**373**	Proquinazid	0.01
64	Chlorantraniliprole	0.01	**219**	Fuberidazole	0.01	**374**	Prosulfocarb	0.01
65	Chlorbromuron	0.01	**220**	Furathiocarb	0.01	**375**	Prothioconazole	0.01
66	Chlorbufam	0.01	**221**	Halosulfuron-methyl	0.01	**376**	Prothiofos	0.05
67	Chlordane-cis	0.01	**222**	Haloxyfop	0.01	**377**	Pymetrozine	0.01
68	Chlordane-trans	0.01	**223**	Haloxyfop-etotyl	0.01	**378**	Pyraclostrobin	0.01
69	Chlorfenapyr	0.01	**224**	HCH-alpha	0.05	**379**	Pyraflufen-ethyl	0.01
70	Chlorfenvinphos	0.01	**225**	HCH-beta	0.05	**380**	Pyrazophos	0.01
71	Chlorfluazuron	0.01	**226**	HCH-delta	0.05	**381**	Pyrazosulfuron Ethyl	0.01
72	Chloridazon	0.01	**227**	HCH-gamma (Lindane)	0.05	**382**	Pyrethrins	0.01
73	Chlorobenzilate	0.01	**228**	Heptachlor	0.05	**383**	Pyridaben	0.01
74	Chlorothalonil	0.05	**229**	Heptachlor-endo-epoxide	0.01	**384**	Pyridalyl	0.01
75	Chloroxuron	0.01	**230**	Heptachlor-exo-epoxide	0.01	**385**	Pyridaphenthion	0.01
76	Chlorpropham	0.05	**231**	Heptenophos	0.01	**386**	Pyrifenox	0.01
77	Chlorpyrifos	0.01	**232**	Hexachlorobenzene (HCB)	0.01	**387**	Pyrimethanil	0.01
78	Chlorpyrifos-methyl	0.01	**233**	Hexaconazole	0.01	**388**	Pyriproxyfen	0.01
79	chlorsulfuron	0.01	**234**	Hexazinone	0.01	**389**	Pyroxsulam	0.01
80	Chlorsulfuron-dimethyl	0.01	**235**	Hexythiazox	0.01	**390**	Quinalphos	0.01
81	Chlorthiophos	0.01	**236**	Hymexazol	0.05	**391**	Quinmerac	0.01
82	Chlozolinate	0.01	**237**	Imazalil	0.01	**392**	Quinoxyfen	0.01
83	Chromafenozide	0.01	**238**	Imazamethabenz-methyl	0.01	**393**	Quintozene	0.01
84	Cinidon-ethyl	0.01	**239**	Imibenconazole	0.01	**394**	QuizaIofop-ethyl	0.01
85	Cinosulfuron	0.01	**240**	Imidacloprid	0.01	**395**	QuizaIofop-P-ethyl	0.01
86	Clethodim	0.01	**241**	Indoxacarb	0.01	**396**	Rimsulfuron	0.01
87	Clodinafop free acid	0.01	**242**	lodosulfuron-methyl Sodium	0.01	**397**	Rotenone	0.01
88	Clodinafop-propargyl	0.01	**243**	lprobenfos	0.01	**398**	Sebuthylazine	0.01
89	Clofentazine	0.01	**244**	Iprodione	0.01	**399**	Sebuthylazine-desethyl	0.01
90	Clomazone	0.01	**245**	Iprovalicarb	0.01	**400**	Simazine	0.01
91	Cloquintocet-mexyl	0.01	**246**	Isazofos	0.01	**401**	Simetryn	0.01
92	Clothianidin	0.01	**247**	lsofenphos	0.01	**402**	Spinetoram	0.01

Table 1. (continued)

93	Coumaphos	0.01	**248**	Isofenphos-methyl	0.01	**403**	Spinosad	0.01
94	Coumatetralyl	0.01	**249**	Isofenyhos-oxon	0.01	**404**	Spirodiclofen	0.01
95	Cyanophos	0.01	**250**	lsoprothiolane	0.01	**405**	Spirornesifen	0.05
96	Cyantranilirpole	0.01	**251**	Isoproturon	0.01	**406**	Spirotetramate	0.01
97	Cyazofamid	0.01	**252**	Isoxaben	0.01	**407**	Spiroxamine	0.01
98	Cycloheximide	0.01	**253**	Karbutilate	0.01	**408**	Sulcotrione	0.01
99	Cycloxydim	0.01	**254**	Kresoxim- methյ	0.01	**409**	Sulfotep	0.01
100	Cyflufenamid	0.01	**255**	Lenacil	0.01	**410**	SulfoxafIor	0.01
101	Cyfluthrin	0.01	**256**	Linuron	0.01	**411**	Sulfur	0.05
102	Cyhalofop-butyl	0.05	**257**	Lufenuron	0.01	**412**	Tebuconazole	0.01
103	Cyhalothrin-Lambda	0.01	**258**	Malaoxon	0.01	**413**	Tebufenozide	0.01
104	Cymiazole	0.01	**259**	Malathion	0.01	**414**	Tebufenpyrad	0.01
105	Cymoxanil	0.01	**260**	Mandipropamid	0.01	**415**	Tebutam	0.01
106	Cypermethrin	0.01	**261**	Mecarbam	0.01	**416**	Tebuthiuron	0.01
107	Cyproconazole	0.01	**262**	Mefenacet	0.01	**417**	Tecnazene	0.05
108	Cyprodinil	0.01	**263**	Mefenpyr-diethyl	0.01	**418**	Tefluthrin	0.01
109	Cyromazine	0.01	**264**	Mepanipyrim	0.01	**419**	TEPP-O,S	0.05
110	Dazomet	0.05	**265**	Mepronil	0.01	**420**	Tepraloxydim	0.01
111	DDD-o,p'	0.01	**266**	Mesosulfuron-methyl	0.01	**421**	Terbufos	0.01
112	DDD-p,p'	0.01	**267**	Metaflumizone	0.0t	**422**	Terbumeton	0.01
113	DDE-p,p'	0.01	**268**	Metalaxyl	0.01	**423**	Terbuthylazine	0.01
114	DDT-o,p'	0.05	**269**	Metaldehyde	0.05	**424**	Terbutryn	0.01
115	DDT-p,p'	0.05	**270**	Metamitron	0.01	**425**	Tetrachlorvinphos	0.01
116	DEET	0.01	**271**	Metazachlor	0.01	**426**	Tetraconazole	0.01
117	Deltamethrin	0.01	**272**	Metconazole	0.01	**427**	Tetradifon	0.01
118	Demeton-S-methyl	0.01	**273**	Methabenzthiazuron	0.01	**428**	Tetramethrin	0.01
119	Demeton-S-methyl sulfone	0.01	**274**	Methacrifos	0.01	**429**	Thiabendazole	0.01
120	Desmedipham	0.01	**275**	Methamidophos	0.01	**430**	Thiacloprid	0.01
121	Diafenthiuron	0.01	**276**	Methidathion	0.01	**431**	Thiamethoxam	0.01
122	Diazinon	0.01	**277**	Methiocarb	0.01	**432**	Thifensulfuron-methyl	0.01
123	Dichlobenil	0.01	**278**	Methiocarb Sulfone	0.01	**433**	Thiobencarb	0.01
124	Dichlofenthion	0.01	**279**	Methiocarb Sulfoxide	0.01	**434**	Thiocyclam Hydrogen Oxalate	0.01
125	Dichlofluanid	0.01	**280**	Methomyl	0.01	**435**	Thiodicarb	0.01
126	Dichlorvos	0.01	**281**	Methoprotryne	0.01	**436**	Thiofanox	0.01
127	Diclofop-methyl	0.01	**282**	Methoxychlor	0.05	**437**	Thiometon	0.01
128	Dicloran	0.05	**283**	Methoxyfenozide	0.01	**438**	Thiophanate-methyl	0.01
129	Dicofol	0.01	**284**	Metobromuron	0.01	**439**	Tolclophos-methyl	0.01
130	Dicrotophos	0.01	**285**	Metolachlor	0.01	**440**	Tolfenpyrad	0.01
131	Dieldrin	0.01	**286**	Metosulam	0.01	**441**	Tolylfiuanid	0.01
132	Diethofencarb	0.01	**287**	Metoxuron	0.01	**442**	Tralkoxydim	0.01
133	Difenoconazole	0.01	**288**	Metrafenone	0.01	**443**	Triadirnefon	0.01

Table 1. (continued)								
134	Diftufenican	0.01	**289**	Metribuzin	0.01	**444**	Triadimenol	0.01
135	Dimethachlor	0.01	**290**	Metsulfuron-methyl	0.01	**445**	Triallate	0.01
136	Dimethenamid	0.01	**291**	Mevinphos	0.01	**446**	Triasulfuron	0.01
137	Dimethoate	0.01	**292**	Milbemectin A3	0.01	**447**	Triazophos	0.01
138	Dimethomorph	0.01	**293**	Milbemectin A4	0.01	**448**	Tribenumn-methyl	0.01
139	Dimoxystrobin	0.01	**294**	Mirex	0.01	**449**	Trichlorfon	0.01
140	Diniconazole	0.01	**295**	Molloniate	0.01	**450**	Triclopyr butotyl	0.01
141	Dinotefuran	0.01	**296**	Monocrotophos	0.01	**451**	Tricyclazole	0.01
142	Diphacinone	0.01	**297**	Monolinuron	0.01	**452**	Trietazine	0.01
143	Diphenamid	0.01	**298**	Monuron	0.01	**453**	Trifloxystrobin	0.01
144	Diphenylamine (DPA)	0.01	**299**	Myclobutanil	0.01	**454**	Triflumizole	0.01
145	Disulfoton sulfone	0.01	**300**	Napropamide	0.01	**455**	Triflumuron	0.01
146	Disulfoton sulfoxide	0.01	**301**	Neburon	0.01	**456**	Trifluralin	0.01
147	Disulfoton	0.01	**302**	Nicosulfuron	0.01	**447**	Triazophos	0.01
148	Diuron	0.01	**303**	Nitenpyrarm	0.01	**457**	Triforine	0.05
149	DMST	0.01	**304**	Novaluron	0.01	**458**	Triticonazole	0.01
150	Dodemorph	0.01	**305**	Nuarimol	0.01	**459**	Vemidothion	0.01
151	Dodine	0.01	**306**	Ofurace	0.01	**460**	Viclozolin	0.01
152	Edifenphos	0.01	**307**	Omethoate	0.01	**461**	Zozamide	
153	Emamectin	0.01	**308**	Ortho Phenylphenol (OPP)	0.01	-	-	-
154	Endosulfan-alpha	0.05	**309**	Orthosulfamuron	0.01	-	-	-
155	Endosulfan-beta	0.05	**310**	Oxadiargyl	0.01	-	-	-
***LOQ: (Limit of Quantification)**								

4. Determination of pesticide residues in the blood samples:

Samples were analyzed in the Central Pesticide Residue and heavy metals Lab. (QCAB) in Giza, Egypt. QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction method followed by high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) was used for the determination of pesticide residues. Blood samples (5 ml) were added to 50 ml polypropylene tube followed by the addition of 5 ml deionized water. This mixture was mixed with vortex (5 s) and left for 10 min for sample hydration. Ten milliliters of acetonitrile were added and mixed with the sample by intensive shaking. Then buffer-salt mixture was added, and the sample was shaken. Centrifugation was carried out for 5 min at 4000 rpm, and the filtration of an aliquot was done with 0.45 µm syringe filter into

2 mL clear HPLC vials. About 1 µL of each sample was injected directly into the HPLC and GC-MS/MS system.

4.1. Determination of pesticide residues by HPLC:

Agilent 1260 Series instrument (HPLC) was used for separation coupled to an API 6500 Qtrap tandem mass spectrometer from AB Sciex with electrospray ionization (ESI) interface. C18 column was used for separation (ZORBAX Eclipse XDB-C18 4.6 × 150 mm, 5µm particle size) (Agilent, USA). The mobile phase was as follows: Solvent A: 10 mM ammonium format solution at pH 4 ± 0.1 in methanol–water (1:9); Solvent B: methanol. The linear gradient program was start at 100% A; 0–13 min from 100% to 5% A; 13–21 min 5% A; 21–28 min from 5%to 100% A; 28–32 min 100% A at a flow rate of 0.3 ml/min. The source was adjusted in the positive mode while nitrogen nebuliser, curtain and other gas parameters were optimized according to the manufacturer recommendations. A source temperature (400°C) and ion spray potential (5500 V) was common for all compounds. Decluster potential and collision energy was in tune by injecting direct infusion from individual pesticide solutions into MS detector. Multiple reactions monitoring mode was used for quantitation and confirmation (Li et al., 2020).

4.2. Determination of pesticide residues by GC-MS/MS:

Gas Chromatography-Mass Spectrometry (GC-MS) measurements were completed using an Agilent Gas Chromatograph system 7890A equipped with tandem mass spectrometer 7000C series GC. Includes the triple Quadrupole GC/MS EI mainframe, EI ion source, ion gauge controller with Routine femto-gram-level limits of detection and quantitation Ultra-low noise, Superior selectivity, Inlet: Split less, Carrier gas: Helium with flow rate 1.830 ml/ minute. Agilent Technologies: HP-5 MS capillary column (5% biphenyl to 95% dimethyl siloxane). Column Internal Diameter (ID): 0.25 mm, Film thickness: 0.52 µm, Column length: 30 m. The initial oven temperature of 70 °C for 2 min, heating from 70 to 150 °C at 25 °C /min, heating from 150 to 200 °C at 3 °C/ min, heating from 200 to 280 °C at 8 °C/min, holding for 10 minutes. The total run time will be 42 minutes.

Quantification of the pesticides was performed by comparing the peak areas of the pesticides to a calibration curve of the standards, and multitude point calibration was used.

4.3. Quality assurance:

An analytical method and instruments were carefully validated as a part of the laboratory quality assurance system and were audited and accredited by the Center of Metrology and Accreditation Finnish Accreditation Service (FINAS) ISO/IEC Guide 17025. The criteria of quality assurance were followed to determine the performance of the standard method. The average recoveries tests on different types of pesticides at different concentration levels varied between 70- 120%. The reproducibility expressed as relative standard deviation was less than 25%. The limit of quantification started at 0.01 mg/kg and up depending on the pesticide type and detected module. The measurement uncertainty expressed as expanded uncertainty and in terms of relative standard deviation (at 95% confidence level) is lower than the default value set by EU (European Union) (± 50%). Blank samples were fortified with the pesticides mixture and analyzed as a normal sample with set of samples. The results were recorded on control charts. Repeated analysis of old samples was regularly carried out to control reproducibility.

RESULTS AND DISCUSSION

Humans are exposed to pesticides through either occupational (pesticide manufacturing/formulation and application in agricultural fields) or non-occupational methods (pollution of the ecosystem through the food chain). A total of 30 blood samples were analyzed for detection of pesticides using multi pesticide residue method to determine the presence or absence of pesticide in occupationally exposed workers. Ten pesticides among 461 pesticides were analyzed (one herbicide, four insecticides, four fungicides and one pesticide synergist) were detected. Data in (Table 2) shown that the most frequently pesticide found in blood samples of spray workers was chlorpyrifos (in 7 blood samples) with percent 35% followed by malathion (in ٤ blood samples) with percent 20%. The highest concentration of pesticide residue in analyzed spray workers blood samples was reported for pyrimethanil fungicide with 0.035 mg/kg in blood samples from Quweisna followed by DDE-p,p' and ortho Phenylphenol with 0.005 in blood samples from Shibin El-Kom.

Data in Figure 1. revealed that the concentration of the fungicide Pyrimethanil residue was the highest in all detected blood samples (0.085 mg/kg), followed by ortho Phenylphenol (0.02 mg/kg) and the lowest was metalaxyl (0.0002 mg/kg).

Chlorpyrifos was detected in 85 percent of the samples examined by Hayat et al., (2010), indicating regular and extensive exposure to this pesticide. Chlorpyrifos concentrations were extremely low as compared to those observed by Schafer et al., (2004), who found that residue levels of chlorpyrifos and methyl parathion in blood and urine samples were up to 4.5 times greater than what the US government considers acceptable, they also reported that two chemicals found in nearly all the test subjects were TCP, a metabolite of the insecticide chlorpyrifos (found in 93% of tested populations), and p,p-DDE, a breakdown product of DDT (found in 99% of tested populations). Our results are in accordance with those obtained by Shalaby and Abdou, (2020) who reported that organophosphate insecticide chlorpyrifos was found in 38.3 percent of blood samples from the study population (farmers, pesticide dealers, and pesticide sprayers) with a concentration of 3.78 mg, followed by acetamiprid (11.7 percent) with a concentration of 1.24 mg, and fenvalerate (1.3 percent) with a concentration of 0.01 mg. They also stated that the presence of Chlorpyrifos pesticide could be due to the fact that it is the most extensively used pesticide in the research area (38.9 percent).

Table 2. The concentrations of pesticide residues detected in blood samples of agricultural spray-workers in Menoufia governorate

Pesticides	Uses	RT* (min)	City Inhabitants (control)			Spray Workers (treatment)		
			n**	range	average	n	range	average
Shibin El-Kom								
Atrazine	H	6.34	ND	ND	ND	0	ND	ND
Chlorpyrifos	I	12.7	1	0-0.000057	0.000057	1	0-0.00128	0.00128
Cyhalothrin-Lambda	I	13.1	0	ND	ND	1	0-0.00168	0.00168
DDE-p,p'	I	14.4	0	ND	ND	1	0-0.005	0.005
Malathion	I	9.38	0	ND	ND	1	0-0.00087	0.00087
Metalaxyl	F	7.04	0	ND	ND	1	0-0.0001	0.0001
Ortho Phenylphenol	F	17.8	2	0.005-0.01	0.0075	1	0-0.005	0.005
Piperonyl butoxide	S	12.5	0	ND	ND	1	0-0.00103	0.00103
Propiconazol	F	11	0	ND	ND	1	0-0.00104	0.00104
Pyrimethanil	F	18.07	1	0-0.01	0.01	1	0-0.005	0.005
Tala								
Atrazine	H	6.34	ND	ND	ND	0	ND	ND
Chlorpyrifos	I	12.7	1	0-0.000057	0.000057	2	0.00057-0.00068	0.000625
Cyhalothrin-Lambda	I	13.1	0	ND	ND	0	ND	ND
DDE-p,p'	I	14.4	0	ND	ND	0	ND	ND
Malathion	I	9.38	0	ND	ND	2	0.00067-0.00146	0.00107
Metalaxyl	F	7.04	0	ND	ND	1	0-0.0001	0.0001
Ortho Phenylphenol	F	17.8	2	0.005-0.01	0.0075	0	ND	ND
Piperonyl butoxide	S	12.5	0	ND	ND	0	ND	ND
Propiconazol	F	11	0	ND	ND	0	ND	ND
Pyrimethanil	F	18.07	1	0-0.01	0.01	0	ND	ND
Quweisna								
Atrazine	H	6.34	0	ND	ND	2	0.00264-0.01168	0.00716
Chlorpyrifos	I	12.7	1	0-0.000057	0.000057	4	0.00057-0.001	0.000713
Cyhalothrin-Lambda	I	13.1	0	ND	ND	0	ND	ND
DDE-p,p'	I	14.4	0	ND	ND	0	ND	ND
Malathion	I	9.38	0	ND	ND	1	0-0.00554	0.00554
Metalaxyl	F	7.04	0	ND	ND	0	ND	ND
Ortho Phenylphenol	F	17.8	2	0.005-0.01	0.0075	0	ND	ND
Piperonyl butoxide	S	12.5	0	ND	ND	1	0-0.00129	0.00129
Propiconazol	F	11	0	ND	ND	1	0-0.00098	0.00098
Pyrimethanil	F	18.07	1	0-0.01	0.01	2	0.01-0.06	0.035

*RT: Retention Time, **n: number of samples (control=10, Shbin El-kom=8, Tala=7, Quwesina=5), ND: Not Detected, H: Herbicide, I: Insecticide, F: Fungicide, S: Synergist.

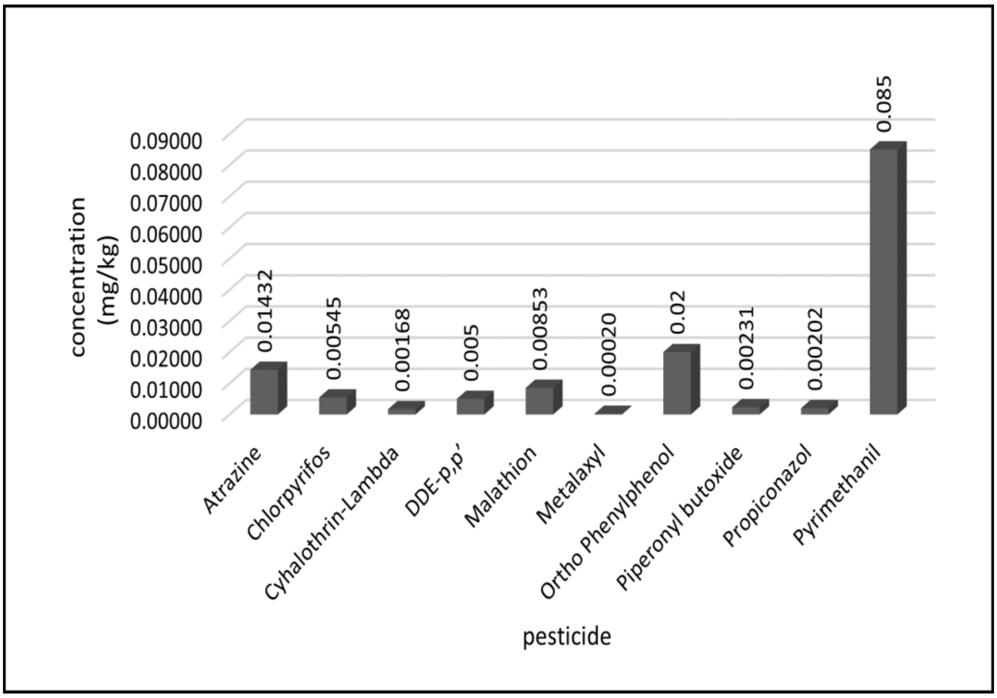

Figure 1. The total concentration of detectable pesticide residues (mg/kg) in the collected blood samples of agriculture spray-workers in Menoufia governorate

populations free from pesticide residues may have used protective equipment before pesticide applications or the detoxification process was caused by e.g., excretion (Usui et al. 2012). Our findings are consistent with those of Arshad et al., (2016), who found malathion residues in 72 percent of all blood samples from pesticide manufacturing workers (ranging from 0.01 to 0.31 mg/l) and Huen et al., (2012), who found levels of diazinon and chlorpyrifos pesticides in the umbilical cord plasma of wombats ranging from 0.0-0.5 ng/mL and 0.0-1.726 ng/mL

Osesua et al., (2018) reported that a major metabolite of DDT, 2,2-bis (p-chlorophenyl)-1,1-dichloroethylene (pp' DDE) was detected in 32 samples at levels of 0. 0338ng/g and have 15.41% distribution in the whole blood samples analyzed. The presence of DDE is because DDE is more persistent than DDT. They also found that among the organophosphorus residues, chlorpyrifos, one of the most extensively used pesticides in households and agriculture, was detected in 100% of the whole blood samples tested, with a mean concentration of 0.0355ng/g and a percentage distribution of 16.18%.

CONCLUSION

In conclusion, the current study found that the population investigated (spray workers and city residents) were occupationally and environmentally exposed to pesticides as a result of the widespread and indiscriminate use of pesticides for pest management. According to this study, human pesticide residue is a biological index/number of pesticide exposure, and blood sample studies may be used to estimate pesticide residues in occupationally exposed and non-exposed people. Our present results indicated that among 461 pesticides analyzed, ten pesticide residues, one herbicide (atrazine), four insecticides (chlorpyrifos, cyhalothrin-lambda, DDE-p,p’ and malathion), four fungicides (metalaxyl, ortho Phenylphenol, propiconazol and pyrimethanil) and one pesticide synergist (piperonyl butoxide) were detected in blood samples of 30 agriculture spray-workers from three different locations in Menoufia governorate. Therefore, intensive training and efforts are required to raise awareness of critical safety practices and to change workers' potentially detrimental environmental behavior. Also, the role of government guidelines and agricultural research services with the cooperation of pesticide manufacturers is very important for reducing pesticide hazards and risks.

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