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Risk evaluation of occupational exposure of southern Brazilian flower farmers to pesticides potentially leading to cholinesterase inhibition and metals exposure

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ABSTRACT

This work presents a frequency matched observational study comparing flower farmers exposed to pesticides and unexposed individuals as controls. All subjects were interviewed before plasma and urine collection. Manganese and Zinc were measured in theses samples by using dynamic reaction cell inductively coupled mass spectrometry. Cholinesterase activity was analyzed through spectrophotometry by using a modified version of the Ellman method. Seventy-eight percent of subjects reported occupational contact with pesticides, from which 37% reported exposure for over 9 years. Flower farms farmers had increased odds of having headache and irritability, respectively, by factors of 6.2 and 2.4 than the control subjects. While the odds of exposed subjects to have insomnia was smaller than control subjects by a factor of 0.34. Exposure to pesticides had a significant effect regarding the plasmatic plasma and urinary manganese levels and whole blood cholinesterase activity ($p < 0.05$). High levels of plasma and urinary manganese, as well as cholinesterase inhibition in whole blood, were evident in the flower farmers who participated in the study.

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1. Introduction

Brazil is considered one of the largest consumers of pesticides worldwide (Moraes, 2019). The environmental and socioeconomic impacts caused by using pesticides are well known. They comprise contamination of water networks, deforestation, inadequate use of the soil, and increased pollution. Associated with these factors is the damage to human health, due to indiscriminate and inappropriate use (Mannion and Morse, 2013). Although the main reports of pesticide poisoning refer to acute events, chronic exposure to these compounds has also shown long-term disorders in the studied group (Gangemi et al., 2016; Li et al., 2019).

Among the several pathologies caused by exposure to pesticides, due to neurotoxic action, neurotoxicity stands out (Mostafalou and Abdollahi, 2017; Furman et al., 2019; Gunnarsson e Bodin, 2019; López-Gálvez et al., 2019). Fungicides containing metals such as Manganese (Mn) and Zinc (Zn) and cholinesterase inhibitors insecticides of the organophosphate and carbamate classes, can cause, in the long term, decreased motor ability, parkinsonism, behavioral and cognitive disorders, among other neurological disorders. Flower crops require the intense use of pesticides to guarantee that the flowers have an expected standard quality for retail. Also, this type of culture does not present the same strictness for waste control as in food agriculture. The classes of pesticides most used to control insects and diseases in flower culture are insecticides and fungicides (Gasparini and Freitas, 2013).

In Brazil, occupational exposure to pesticides in vulnerable groups, such as family farmers, the limited literature on the long-term damage caused by exposure, added to the difficulty of accessing information and health care for this group, show the complexity and relevance of the theme. It is worth noting that family farming is an important source of income in the region of this study and that we have identified in preliminary studies, a migration of wine makers followed by our research group to flower farming, which encouraged us to conduct this research. The cultivation of flowers is conductive to pesticides exposure use of these products to achieve high-quality standards for commercialization (Gasparini and Freitas, 2013).

Characterization of occupational exposure of flower crop workers in greenhouses (environments with high temperatures and humidity, besides being confined spaces, which contributes to an increase in the risk of exposure) has evidenced the presence of neurological, respiratory, and dermal problems (Hanssen et al., 2015; Ribeiro et al., 2012; Shentema et al., 2020). However, there is a lack of information on outdoors work, which, despite not offering risk factors related to confined environments, still offers health risks.

Evaluation of exposure to a single pesticide or class, as it is currently done, underestimates the real health risks workers are submitted to when using different compounds that may result in unknown effects. Furthermore, inadequate and inefficient control associated with unsafe cultivation practices increase intoxication rates (Breilh et al., 2012). As chronic pesticide poisoning is challenging to diagnose, we are looking for data capable of underlining the need for greater care for the health of the group in question. Therefore, cholinesterase activity and both plasma and urinary levels of Manganese and Zinc of flower farmers in a municipality in the south of Brazil were determined and statistically calculated to observe possible correlations with the symptoms developed with the use of these compounds.

2. Material and methods

This is a frequency matched observational study comparing flower farmers and unexposed individuals, as controls, carried out from December 2018 to August 2019 in the municipality of Marialva, Paraná, Southern Brazil. The samples were collected from flower farmers through convenience sampling. The first contact was intermediated by the Institute of Rural Development of Paraná (IDR Paraná). According to our inclusion criteria, participants had to be adults, either males or females, family farming flowers, working with flower crops for over 1 year. The visits to family farmers were previously scheduled to enable the interviews and biological material collection. Blood and urine sample collection took place at the family farmers' houses. Sampling was carried out before the beginning of the morning work to avoid contamination through working clothes. All the blood samples were placed in 6.0 ml heparinized metal-free tubes for vacuum collection of the VACUETTE® brand (C180935W, Brazil) by venipuncture in the cubital fossa. They were used to determine cholinesterase activity through spectrophotometry by using a modified version of the Ellman method. Urine samples were self-collected by the volunteer through spontaneous urination. To that end, workers were asked to wash their hands before collection, and metal-free flasks were used. After collection, both urine and blood samples were sent to laboratory for processing. Blood samples were centrifuged at 2000 rpm for 10 min to separate plasma from other blood elements. Both sample types (urine and plasma) were fractioned in Eppendorf™ metal-free tubes for Mn and Zn analysis. An enzymatic colorimetric method, by Analisa™, was used to determine the urinary creatinine value for each individual. Creatinine value was used to correct possible variations in elements concentration that may be affected by urine volume.

The participants were asked some questions through an in-person interview carried out by a trained interviewer. The questionnaire was developed according to the Roadmap for Chronic Pesticide Poisoning Evaluation of Paraná State Department of Health (SESA, 2013), with information related to sex, age, use of PPE, type of contact with pesticides, exposure time, symptoms related to chronic exposure, and others. This roadmap is a reference guide in Paraná for groups exposed to pesticides.

An occupationally unexposed group was chosen because it was not possible to keep the flower farmers away from exposure to use them as their own control group for laboratorial analysis. The control group comprised individuals who had never done farm work and had never been exposed to pesticides in an occupational context. This group consisted of blood donors from the Regional Blood Centre of Maringá. Samples from healthy blood donors were collected shortly after the exposed workers had their samples collected. The volunteers also filled out a simple form with information regarding age, sex, occupation, and place of origin. The laboratory analyzes were performed in the same way in the exposed and non-exposed groups. The unexposed individuals were selected based on the age and gender profile of the flower farmers participating in the study. We performed a statistical comparison to assess the study groups concerning their comparability with respect to gender and age and we included these variables as potential confounders in all analyses (Supplementary Material I). Flower farmers and the unexposed group did not live in the same area. The Regional Blood Centre, from where volunteers were recruited to compose the non-exposed group, is about 19 miles away from the flower crops studied in this research.

Of these voluntaries, 68 were included in the analysis after application of the same exclusion criteria used for the group exposed to pesticides. All participants (exposed and unexposed) signed an informed consent form. The study was approved by the Standing Committee for Ethics in Research Involving Human Beings (COPEP) of the State University of Maringá, report no 3.314.720.

2.1. Quantification of metals in plasma and urine by inductively coupled plasma mass spectrometer (ICP-MS)

The measurement of Mn and Zn in plasma and urine was performed at the Federal University of ABC Paulista (UFABC - Santo André-SP). For the measurement of metals, the samples were homogenized and diluted (1:10 - v/v) in a solution with 0.4% (v/v) nitric acid (HNO3) and 0.005% Triton X-100 (m/v). To calibrate the equipment, a matrix combination (plasma and urine) was used in 15 ml conical tubes of the Falcon® brand. The analytes were added in 0.4% (v/v) HNO_3 and 0.005% (m/v)

Triton X-100 containing matrix (1:20 - v/v). The samples were injected in a mass spectrometer with inductively coupled plasma (ICP-MS Agilent 7900, Hachioji, Japan) (Batista et al., 2009a,b; Freire et al., 2018).

2.2. Measurement of cholinesterase levels

To measure cholinesterase in whole blood, the blood samples were aliquoted for evaluation on the day of collection. According to the regulatory standard of the Ministry of Labor NR-7, in force at the time the study was carried out, and which addresses the parameters for biological control of occupational exposure to chemical compounds, sampling is not critical. It means that sample collection may be performed on any day, at any time, as long as the workers have been working for the last 4 (four) weeks, without time off for more than 4 (four) days. The same standard recommended the use of cholinesterase in whole blood as a biological indicator of exposure to organophosphate esters and carbamates (Regulatory standard number 7, 1994) Cholinesterase determined in whole blood offers the advantage of not suffering interference in cases of hemolysis. In addition, it is not necessary to separate the erythrocytes from plasma, which avoids the low precision and sensitivity that occur in analyses with erythrocytes (Tecles et al., 2002). Still, other studies evaluating occupational exposure to pesticides use the determination of cholinesterase in whole blood (Ntow, 2009; Sapbamrer, 2014). By using the technique of Ellman et al. (1961), modified by Harlin and Ross (1990), we evaluated the cholinesterase activity in whole blood. A 10 µl sample of blood heparinized was first diluted with 10 ml of phosphate buffer (pH 8.0) and, then, homogenized. 3 ml of this solution were mixed with 50 µl of dithiobisnitrobenzoic acid and, then, 20 µl of the 5% acetylthiocholine iodide were added to the later solution. Absorbance was measured by using a spectrophotometer visible in the UV (Shimadzu UV-1601PC), with a 412 nm length and 30 °C at 60, 120, 180, 240 and 300 s. Cholinesterase activity (µmols / ml / minute) was then obtained.

2.3. Statistical analysis

In this study, a general linear regression model considering the normal distribution of values was used to assess the effect of gender(G), exposure (E) and age (A) over the continuous variables/outcomes, such as: Mn and Zn levels in urine and plasma, and Cholinesterase (Sarkar, 2008). The A and G variabilities were calculated and removed by using the least square mean (LS mean), that is a calculated mean when the selected covariate of age and gender are assessed and removed. For binary variables, a logistic regression (or logit) model, considering a binomial distribution, was applied. In that case, the log odds (logarithm of the odds) of the outcome were modeled as a linear combination of the predictor variables (supplementary material I and II).

In both regression types considered age (A), gender (G), exposure (E) and type of pesticide were tested by factorial ANOVA. The best model to explain each of the measured variables as a function of the considered factors/effects was identified by AIC and ANOVA. The significance level was 0.05 The complete statistical assessment was performed using R and it is presented in the supplementary material (Marschner, 2011).

3. Results

3.1. Sociodemographic characteristics and data of the studied group

The sample consisted of 109 participants, 41 individuals exposed to pesticides and 68 unexposed ones (control group). Most of them were men, 63% in the exposed group and 57%in the control group. The flower farmers were, on average, 46.9 years old (± 12.33), while the members of the control group were 38.4 years old (± 12.94) on average. As for alcohol consumption, 49% of the flower farmers reported the habit of drinking alcohol, whereas 60% of the participants from the control group reported consuming it. As for tobacco use, only members of the group exposed to pesticides reported the habit of smoking, where 7% claimed to be smokers and 24% ex-smokers (Table 1). There were no smokers among the volunteers of the control group.

Regarding the type of exposure to pesticides, 78% of the interviewees reported contact at work, with 36% having been exposed occupationally for more than 9 years. As for the use of personal protective equipment (PPE), 74% of the flower farmers stated they made use of some equipment, 6% claimed that they used all the recommended PPE and 19% declared not to use any PPE. Table 1 presents the full data.

The main types of contact with pesticides by flower farmers are at harvesting (66%), during application of pesticides (54%), transport (54%), equipment cleaning (49%), pesticide preparation (46%) and storage (46%).

The flower farmers reported the use of 15 pesticides, arranged by their chemical name in Table 2. Among these pesticides, 53% are fungicides, 40% are insecticides and 7% are acaricides.

3.2. List of symptoms of the studied group

Table 3 outlines the clinical symptoms self-reported by flower farmers and unexposed controls. The data showed a predominance of symptoms that can point to neurological problems: irritability (56%), numbness (32%), headache (29%), dizziness (24%), insomnia (22%), excessive sweating (22%), tremors (10%) and lack of appetite (5%).

Table 3 shows the association between the self-reported symptoms of the exposed and unexposed groups, separated by gender, and odds ratio by factor. The results were modeled as a linear combination of the predictor variables (exposure, gender and age). Exposed individuals present increased odds of having headache and irritability by a factor of 6.17 and 2.4 respectively when compared with the odds of control subjects. As well as the odds of exposed subjects having insomnia were reduced by a factor of 0.34 when compared to the odds of unexposed ones. The results also show that men's odds of having headaches (OR=0.20), vertigo (OR=0.36) and insomnia (OR=0.45) were reduced when compared with women's odds.

3.3. Measurement of cholinesterase levels

Data collection (Table 2) revealed the use of cholinesterase inhibitor insecticides (Formethanate hydrochloride and chlorpyrifos). Due to the mechanism of action of these pesticides, the analyzes were carried out for the measurement of cholinesterase in whole blood, the results were

Table 1
General characteristics of groups exposed and unexposed to pesticides, Marialva state of Paraná, Brazil, 2019.

	Exposed (n = 41)	Unexposed (n = 68)
Sex(N (%))		
Male	26(63)	39(57)
Female	15(37)	29(43)
Age (mean±SD)	46.95(± 12.33)	38.45(± 12.94)
Alcohol consumption(N (%))		
Yes	20(49)	41(60)
No	21(51)	27(40)
Smoker (N (%))	3(7)	–
Ex-smoker (N (%))	10(25)	–
Non-smoker (N (%))	28(68)	–
Type of exposure (N (%))		
Occupational	32(78)	–
Environmental	9(22)	–
Period of exposure (N (%))		
≤ 9.0 Years	15(43)	–
9.0 — 18.0 Years	8(23)	–
18.0 — 27.0 Years	5(14)	–
≥ 36.0 Years	7(20)	–
Personal Protective Equipment (N (%))		
None	6(20)	–
Some equipment	23(74)	–
All equipment	2(6)	–

Table 2
Percentage of pesticides used on properties by flower farmers, Marialva, state of Paraná, Brazil, 2019.

Chemical name	Type of action	WHO toxicological classification	Percentage of use (%)
Boscalid + kresoxim-methyl	Fungicide	U[1]	26
Folpet	Fungicide	U[1]	39
Avermectin	Inseticide	–	65
Thiophanate-methyl + Chlorothalonil	Fungicide	U[1]	63
Metiram + Pyraclostrobin	Fungicide	U[1]	65
Spiromesifen	Inseticide	II[3]	21
Mancozeb	Fungicide	U[1]	31
Formetanate hydrochloride	Inseticide	Ib[4]	46
Chlorpyrifos	Inseticide	II[3]	12
Cypermethrin	Inseticide	II[3]	34
Thiophanate-methyl	Fungicide	U[1]	68
Fluxapyroxad + Pyraclostrobin	Fungicide	–	19
Imidacloprid	Inseticide	II[3]	24
Sulphur	Acaricide	III[2]	5
Metiram	Fungicide	U[1]	3

Legend: 1Unlikely to present acute hazard; 2Slightly hazardous; 3Moderately hazardous; 4Highly hazardous.

separated by sex (Table 4). The statistical analysis showed a significant effect of exposure to pesticides and sex on cholinesterase activity ($p < 0.05$), and a weak and combined effect concerning age and sex. All results can be found in the supplementary material. The data also showed that enzyme activity in the exposed group, in both men and women, had a decrease.

3.4. Quantification of manganese and zinc in plasma and urine

Regarding the pesticides used (Table 2), the exposed group reported the use of Metiram + Pyraclostrobin and Mancozebe, which contain Mn and Zn in their composition. Thus, the plasma and urinary levels of Mn and Zn was performed (Fig. 1). Some outliers were found for urinary (Subject 27) and plasmatic (subjects 34 and 42) Mn and urinary Zn (subjects 27 and 80). These samples exceeded the average more than 3 times the standard deviation. These values could not be explained by any of the studied factors (A, G and E).

Details of the statistical analysis are presented in the Supplement Material I. A statistically significant effect of exposure to pesticides and gender on plasma and Mn urinary values ($p < 0.05$). The combination of these two effects (G*E) also presented significant effect over Mn levels. Age had no significant effect over Mn levels ($p > 0.05$). The results also showed higher amounts of Mn in the plasma from men and urine from women exposed to pesticides. There was no statistically significant difference between exposed and unexposed subjects observed on Zn urinary and plasmatic levels. Gender and age however significantly affected the Zn concentrations ($p < 0.05$). Cholinesterase levels were significantly affected by gender, exposure and a combination of Age*-Gender ($p < 0.05$). Since there are no universal standard values for exposed and unexposed individuals, a comparison is usually made between exposed and unexposed individuals.

4. Discussion

This study investigated a group of flower farmers occupationally exposed to pesticides, mainly from the classes of fungicides and insecticides. The main findings show the association of clinical symptoms such as headache and irritability with exposure. The data demonstrated cholinesterase inhibited activity, positively related to men and women, besides increased urinary levels of Mn in both sexes, whereas increased plasma level of Mn was only associated with men.

Flower farmers, as well as workers from other agricultural areas, are exposed to occupational risk due to contact with pesticides. Studies have reported evidence on occupational exposure to pesticides and the onset of neurological, respiratory, reproductive disorders, neoplasms, among others (Wirdefeldt et al., 2011; Brouwer et al., 2015).

Individuals chronically exposed to pesticides may develop nervous system pathologies (Ellingsen et al. 2008; Bowler et al., 2011; Burke et al., 2017; Roels et al., 2012; Wasserman et al., 2011; Gill et al., 2011).

Table 4
Comparison of the geometric mean of cholinesterase activity in whole blood (μmols / ml / minute) in the exposed sample and not exposed to pesticides separated by sex.

Sex	Exposed		Unexposed	
	GM[1]	CI95%[2]	GM[1]	CI95%[2]
Male	5.2	± 0.7	6.2	± 1.2
Female	4.7	± 0.9	5.2	± 1.0
	Factor	***p*- value**		
	Age	0.691		
	Sex	**5.184e-06**		
	Exposure	**1.139e-06**		

[1]Geometric Mean [2]Confidence Interval.

Table 3
Comparison between self-reported symptoms in the group exposed and unexposed to pesticides separated by sex.

Symptoms	Exposed (n = 41)		Unexposed (n = 68)		Odds ratio by factor		
	Male (n = 26)	Female (n = 15)	Male (n = 39)	Female (n = 29)	Exposure*	Gender**	Age***
Headache	5	7	1	5	**6.17**	**0.20**	0.99
Vertigo	5	5	10	15	0.61	**0.36**	0.99
Cramps	8	6	15	17	0.45	0.53	1.03
Weakness	6	5	6	7	1.31	0.60	1.03
Lack of appetite	2	0	1	6	0.37	0.32	1.03
Blurred vision	3	3	2	5	0.94	0.35	1.07
Tremors	3	1	3	7	0.68	0.46	1.00
Irritation	14	9	11	13	**2.40**	0.58	1.00
Tingling	9	4	6	10	1.26	0.65	1.03
Insomnia	5	4	11	15	**0.34**	**0.45**	1.04
Nausea	3	0	2	6	0.74	0.51	0.98
Cough	4	1	2	4	1.26	0.80	1.02
Sweating	4	5	8	6	1.02	0.69	1.01
Ocular and nasal disturbances	4	4	7	9	1.00	0.49	1.00
Skin problems	2	2	2	4	0.98	0.42	1.02

*Controls were the reference group, * * females were the reference group, * ** Ages were considered by year. Values in bold indicate statistically significant differences.

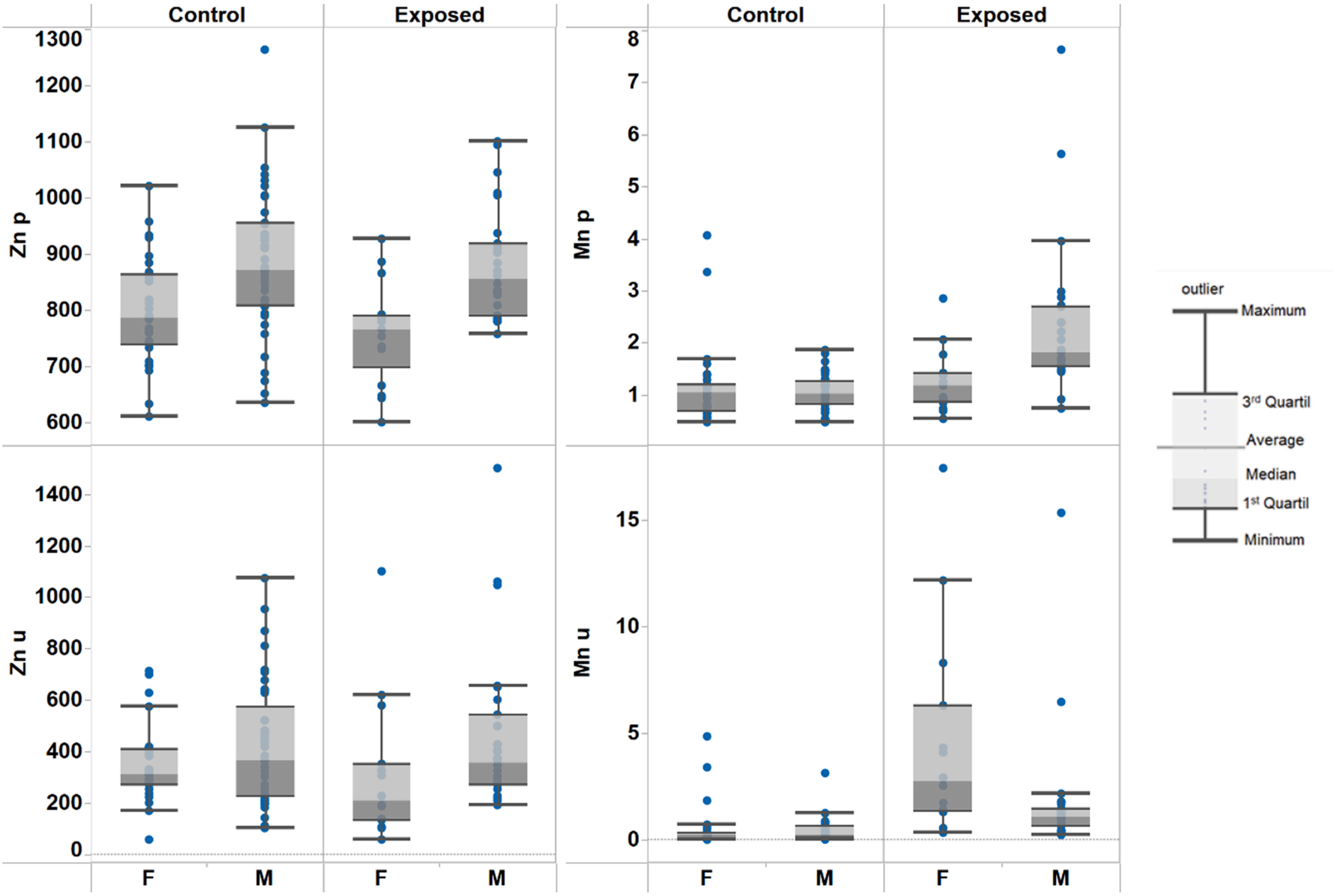

Fig. 1. Boxplot of urinary (u) and plasmatic (p) Zinc and Manganese, in μg/L and μg/g levels by control and exposed subjects and by gender (F= Female and M = Male).

In 2018, Brazil marketed a total of 549,280.44 tons of pesticides (Brazilian Institute of the Environment and Renewable Natural Resources – IBAMA). The fungicide named Mancozebe (composed of Mn and Zn) ranked third (40,549.92 tons), and the insecticide called Chlorpyrifos (cholinesterase inhibitor insecticide) ranked tenth (10,827.78 tons). These data point to the relevance of studying these classes of pesticides and their relationship with workers' health (IBAMA, 2019).

The scientific literature on health monitoring of flower farmers in Brazil and worldwide (Breilh et al., 2012; Ribeiro et al., 2012; Hanssen et al., 2015; Mwabulambo et al., 2018; Shentema et al., 2020) refers only to greenhouse flower farmers. As for outdoors flower crop workers, there is still no information available, until the date of this study. In this research, we presented relevant data on aspects involving occupational exposure of this group of workers.

Compliant with Brazilian law, Regulatory Standard number 6 (2018) states that workers involved in the use and handling of pesticides must be wearing personal protective equipment (PPE), such as gloves, boots, cap, goggles, mask, and other items that may minimize contact with toxic agents. It is believed that long-lasting occupational exposure (> 9 years) of flower farmers to pesticides, with no PPE, may contribute to the onset of the symptoms reported in this study.

The exposure of flower farmers to cholinesterase inhibitor insecticides is also related to neurological disorders (Gill et al., 2011; Naughton and Terry Jr, 2018). It may also be responsible for the changes reported in this study, which are associated with the nervous system (Table 3).

Studies carried out in other countries with flower farmers exposed to pesticides present results aligned to those obtained in this study. Mwabulambo et al. (2018), when addressing a group of flower farmers in Tanzania, showed that a large part of the sample group had neurological symptoms, such as excessive sweating (46.4%), body weakness (34.5%), abnormal tiredness (28.6%) and headache (27.4%). In Ethiopia, another study showed a prevalence of neurological symptoms, such as headache, depression, anxiety, fatigue, and dizziness, in both men and women involved in flower culture (Hanssen et al., 2015).

A research carried out in Brazil, which evaluated occupational safety and health practices of workers in flower greenhouses, reported symptoms such as headache (33%), itching (25%), memory loss (25%), chest pains (17%), and dizziness (17%) (Ribeiro et al., 2012). Breilh et al. (2012), when seeking to validate a methodology to assess chronic exposure to pesticides among flower farmers in Ecuador, identified symptoms such as mucosal irritation (83.6%), irritability (64.2%), headache (59.3%), salivation (43.1%), weakness (40.7%), excessive sweating (38.2%), abdominal pain (36.6%) and dizziness (36.1%).

Regarding self-reported symptoms (Table 3), we found that flower farmers using pesticides have more than six-fold increase in the occurrence of headaches and more than two-fold increase when it comes to irritability, compared to unexposed individuals. Insomnia was also shown to be increased in flower farmers (Table 3). The link between neurobehavioral symptoms, as sleep health, and pesticide exposure among occupationally exposed groups has been documented. These findings are in line with others studies that have demonstrated the association with poor sleep health among farmworkers and cumulative pesticide exposure (Fuhrimann. et al., 2022; Zamora et al., 2021; Li et al., 2019; Serrano-Medina et al., 2019; Baumert et al., 2017; Postuma et al., 2012; Zhao et al., 2010). Short sleep duration reflects highly reactive nerve cells, or nervous stress or disruption, and exposure to pesticides might delay sleep time and reduce sleep duration (Kim, Kabir, Jahan, 2016; Meyer-Baron et al., 2015). Recently, Thammachai et al. (2022) revealed a significantly higher prevalence of neurobehavioral symptoms (OR = 8.99 for insomnia) related to pesticide exposure.

The data also showed that women are more likely to have headaches, vertigo and headaches when compared to men. The full analysis is available in the supplementary material. Other studies carried out in different countries, without reports of the pesticides used, also found evidence of significant higher risk for workers to have other symptoms, such as eye irritation and physical weakness. These symptoms were also evaluated in our study (Table 3), but no significant values were found for them. We can infer that different types of cultures and their respective variability regarding the use of pesticides can contribute to an increase in the occurrence of neurological symptoms (Dasgupta et al., 2007; Sapbamrer and Nata, 2014; Hongsibsong et al., 2017; Sapbamrer et al., 2019).

Mn and Zn, metals that are present in the composition of the fungicides used by flower farmers, are considered essential for the human body (ATSDR, 2005; ATSDR, 2012; Bhattacharya et al., 2016). These essential metals are toxic at high exposure levels, and increasing evidence points to negative health effects from cumulative low-level exposure. Standard values used in routine laboratories are adopted to investigate cases of acute toxicity by these compounds. To assess cumulative exposure, studies on occupational exposure monitoring have been used to compare values of essential elements in exposed groups with groups not exposed to the same compounds. Essential and non-essential metals are present in a higher concentration of biological material from workers who handle pesticides compared to unexposed individuals (Ghazali et al., 2012; Gunier et al., 2013; Mora et al., 2014).

Regarding the quantification of Mn in plasma and urine performed in this research, these are matrices were capable to demonstrating the effects of the contact with pesticides (Smith et al., 2007; Laohaudomchok et al., 2011; Baker et al., 2015). Although studies use matrices such as hair, nails, and saliva, there is still no fully consolidated biological marker to monitor Mn in isolation (O'Neal and Zheng, 2015).

Tables 5 and 6 showed that the exposed individuals present higher average Mn in the plasma and urine when compared to the unexposed group. Plasma levels were nearly twice as high as in unexposed group. Urinary Mn levels were 4 times higher when compared to the control group. Studies that evaluated exposure to pesticides, achieved potentially toxic metal levels, also high when compared to unexposed individuals (Cowan, 2009; Rocha et al., 2015; Zhang et al., 2015).

The literature highlights that there is susceptibility to Mn concerning sex. Women of different ethnicities and from different countries were analyzed, showing higher levels of Mn in plasma and urine than men. The authors propose that metabolic differences between men and women in the regulation of Mn may be related to this difference (Bocca et al., 2011; Lee et al., 2012; Oulhote et al., 2014; Zhang et al., 2015; Smpokou, 2019). In this study, the variables sex and exposure to pesticides showed a significant combined effect ($p < 0.01$) on urinary Mn values, contributing to the average results (Table 6) obtained for the urinary levels of women exposed to pesticides. Our results show that, besides sex susceptibility, the pesticide exposure factor may also change urinary Mn values, showing that there may be other parameters that may impact Mn concentration, such as exposure to pesticides. There was no significant effect of exposure to pesticides in plasma and urinary Zn concentrations. However, the values found are like those obtained in other studies, with groups exposed to pesticides (Jayasumana et al., 2015; Rocha et al., 2015).

Table 5
Comparison of the geometric mean of plasma levels of Manganese and Zinc in µg/L in the samples exposed and unexposed to pesticides separated by sex.

	Exposed		Unexposed	
	GM[1]	CI95%[2]	GM[1]	CI C95%[2]
Manganese				
Male	2.0	±2.1	1.0	±0.5
Female	1.2	±1.0	0.2	±1.6
	Factor	***p*- value**		
	Age	0.616		
	Sex	**0.030**		
	Exposure	**1.564e-05**		
Zinc				
Male	878.0	±141.0	878.0	±174.0
Female	750.0	±155.0	791.0	±141.0
	Factor			
	Age	0.041		
	Sex	**2.08e-05**		
	Exposure	0.83920		

[1]Geometric Mean [2]Confidence Interval.

Table 6
Comparison of the geometric mean of urinary levels of Manganese and Zinc in µg/g creatinine in the samples exposed and unexposed to pesticides separated by sex.

	Exposed		Unexposed	
	GM[1]	CI95%[2]	GM[1]	CI95%[2]
Manganese				
Male	1.0	±4.3	0.2	±0.9
Female	2.7	±8.0	0.2	±1.6
	Factor	***p*- value**		
	Age	0.238		
	Sex	0.051		
	Exposure	**2.44e-6**		
Zinc				
Male	382.0	±405.0	468.0	±328.0
Female	233.0	±454.0	359.0	±206.0
	Factor	***p*- value**		
	Age	0.140		
	Sex	**0.036**		
	Exposure	0.767		

[1]Geometric Mean [2]Confidence Interval.

Monitoring cholinesterase activity in whole blood allowed us to observe that the individuals exposed to inhibitor insecticides showed a reduction of enzymatic activity in both sexes (Table 4). The factor 'sex' seems to show important differences for cholinesterase activity in whole blood, with an approximately 15% decrease in men and 8% in women compared with unexposed individuals.

Mwabulambo et al. (2018) studied flower farmers in Tanzania exposed to the same insecticides. They found a significant decrease in enzyme activity. In the same country, in another study performed with a group of flower farmers who reported a reduced use of cholinesterase inhibitor insecticides in their plantations, no decrease in enzyme activity was observed (Shentema et al., 2020). A study carried out in the southern region of Brazil with rural workers assessed the relationship between occupational exposure to pesticides and metals and exposure biomarkers. The authors highlighted that there was a significant decrease in cholinesterase activity among individuals from the exposed group, as well as biological changes. These data may indicate that rural populations, in general, are exposed to the risks of developing pathologies resulting from exposure to pesticides (Cestonaro et al., 2020).

According to the authors, such population is rarely exposed to just one pesticide, and our results have the same pattern (Table 2). Thus, we must consider exposure to pesticide compounds. According to a study by Mwila et al. (2013) who evaluated the effect of carbamates and organophosphates compounds on the activity of the cholinesterase enzyme, the mixture of these pesticides resulted in an additive effect of enzymatic inhibition. Other articles show that pesticide compounds can contribute to DNA damage (Benedetti et al., 2018; Cuenca et al., 2019). These interactions can trigger mechanisms that have not been studied yet, such as the interaction between different pesticides classes, which may result in synergisms or toxicity changes already reported in the literature.

Since the metabolism of metals has an individual variability, we compared it to a group of individuals occupationally not exposed to pesticides, without self-reports of clinical symptoms, since qualifying for blood donation requires an individual to be healthy. Thus, the differentiating factor between the two groups is the absence of occupational exposure and self-reported pathologies and/or symptoms in the group not occupationally exposed to pesticides, thus, serving as a comparison for this sample of a group exposed to pesticides. As this is a cross-sectional study, characterized by the investigation of a specific period with individuals of interest, we can only infer that the increased levels of Mn and Zn, in relation to the group not occupationally exposed, may be related to the self-reported symptoms. However, a retrospective study would be necessary to establish a cause-and-effect relationship.

The symptoms pointed out in this study were extracted from an adapted instrument, developed according to the Roadmap for Chronic Pesticide Poisoning Evaluation of the Paraná State Department of Health (SESA, 2013). That is a reference guide in Paraná for groups exposed to pesticides. In this study, as well as in similar ones, we may not say that the symptoms result from exposure to pesticides. Yet, since this is a cross-sectional study, the sample did show an increased risk for some symptoms.

Some limitations of this study need to be taken into account. There is a lack of information on previous cases of occupational exposure to pesticides with other cultures. Flower crops are located close to other crops, which is an aggravating factor for the exposure of family farmers, who are already a vulnerable group. In this case, cumulative exposure may be underestimated, because not every case of exposure is a result of the current farming process. Controling life habits is another important factor, as there may be exposure to other sources of emission, such as environmental and food exposure, which were not considered in this study. The symptoms reports were obtained at a point in time, based on self-reported information, with the chance of memory bias.

Despite the small sample size, this study offers an important contribution by relating self-reported neurological symptoms from chronic exposures to the laboratory analyses performed, using an integrated approach that the multifaceted problem of pesticide poisoning requires. New studies should be carried out to monitor the group. An option would be a cohort study to identify a cause-and-effect relationship.

5. Conclusion

The findings suggest that flower farmers in the region under study are chronically exposed to pesticides, which may be associated with the clinical symptoms reported by them. Neurological symptoms, high levels of plasma and urinary manganese, as well as cholinesterase inhibition in whole blood were evident in the flower farmers exposed to pesticides. Quantification of plasma and urinary levels of metals and the measurement of cholinesterase in whole blood can assist in the biological monitoring of workers, together with clinical monitoring, technical guidance on PPE usage, thus, helping to prevent and protect flower farmers from future intoxications.

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CRediT authorship contribution statement

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Raul Gomes Aguera, Camila da Silva Freires, Luís Otavio de Oliveira, Renata Sano Lini and Jéssica Cristina Zoratto Romoli. Investigation and methodology were performed by Raul Gomes Aguera, Renata Sano Lini, Jéssica Cristina Zoratto Romoli, Bruna Moreira Freire and Bruno Lemos Batista. Data analysis was performed by Raul Gomes Aguera and Lucilena Rebelo Monteiro. Data curation, project administration, resources and supervision were performed by Samuel Botião Nerilo, Miguel Machinski Junior and Simone Aparecida Galerani Mossini. The first draft of the manuscript was written by Raul Gomes Aguera and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.etap.2022.103874.

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