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RESEARCH ARTICLE

Elemental plasma content and urinary excretion in vineyard farmers occupationally exposed to pesticides in southern Brazil

Renata Sano Lini[1] · Raul Gomes Aguera[1] · Danielle Hoeltgebaum[2] · Fernanda Pollo Paniz[3] · Tatiana Pedron[3] · Silvia Capelari[4] · Lucilena Rebelo Monteiro[5] · Miguel Machinski Junior[2] · Samuel Botião Nerilo[6] · Bruno Lemos Batista[3] · Magda Lúcia Félix de Oliveira[7] · Simone Aparecida Galerani Mossini[1,8]

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Abstract

This is a cross-sectional study with data and biological material collection from vineyard farmers in southern Brazil. An interview was carried out through a questionnaire developed according to the reference guide of the state government. Plasma and urine samples were screened for Aluminum, Chromium, Manganese, Copper, Nickel, Cobalt, Zinc, Arsenic, Selenium, Cadmium, Antimony, Barium, Mercury, Lead and Uranium, with a technique for fast determination of these elemental contents in biological material utilizing dynamic reaction cell inductively coupled mass spectrometry. Principal component analysis was used to identify associations between these elemental contents in biological samples and the information obtained from the interviews. The farmers showed some trace elements in plasma and urine at a higher concentration than unexposed populations from other studies. This study highlights recent findings of trace elements in biological material and their association with characteristics of pesticide use. In addition, it also contributes to the gap in the literature regarding trace elements content in plasma and urine of workers exposed to pesticides.

Keywords Trace elements · Farmers · Viticulture · Pesticides · Occupational exposure · Principal component analysis

Introduction

Pesticides are widely used all over the world to combat pests and diseases with the aim of increasing agricultural productivity. Since 2008, Brazil has been the largest consumer of pesticides in the world (Carneiro, 2015). In 2019, more than 620,000 tons of active ingredients were consumed in Brazil according to the annual bulletin of the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA 2019).

In Brazil, the use of agrochemicals by rural workers often occurs without the use of personal protective equipment (PPE), and this can lead to cases of acute poisoning and health damages (Sousa et al. 2016; Viana et al. 2017). In 2019, 5700 cases of acute agriculture pesticide poisoning were registered in the Notifiable Diseases Information System (DATASUS

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✉ Simone Aparecida Galerani Mossini
sagmossini@uem.br

[1] Postgraduate Program in Biosciences and Physiopathology, State University of Maringá, Maringá, Paraná, Brazil

[2] Postgraduate Program in Health Sciences, State University of Maringá, Maringá, Paraná, Brazil

[3] Center for Natural Sciences and Humanities, Federal University of ABC (UFABC), Santo André, São Paulo, Brazil

[4] Paraná Rural Development Institute (IDR - PR), Marialva, Paraná, Brazil

[5] Centro de Química e Meio Ambiente (CEQMA), Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN-SP), São Paulo, Brazil

[6] Ingá University Center (Uningá), Maringá, Paraná, Brazil

[7] Poison Control Center at Maringá University Hospital, Maringá, Paraná, Brazil

[8] Department of Health Basic Sciences, Laboratory of Toxicology, State University of Maringa, Avenue Colombo no 5790, Maringa, PR 87020-900, Brazil

2019). Furthermore, despite the clinical importance of acute poisoning, several studies report damage to health of rural workers that could be related to chronic exposure to pesticides (Carneiro 2015; SESA 2013).

Some elements like Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb), Manganese (Mn), Nickel (Ni), Cobalt (Co), Zinc (Zn), and Copper (Cu) are common contaminants in pesticides widely used in rural areas (Alkhader 2015; Cestonaro et al. 2020; Gimeno-García et al. 1996; Rocha et al. 2015). In addition, although there are few reports on the presence of Selenium (Se) and Mercury (Hg) in pesticides, these elements can be present in the environment and have toxicological importance (Buschinelli 2020; Nordberg et al. 2014; Vinceti et al. 2012).

Metals and metalloids are xenobiotics that could be present as environmental (water, soil, air, and food) contaminants, especially in rural areas (Nordberg et al. 2014). For example, aluminum (Al) is the third most abundant element in the Earth's crust, and studies confirm that it can cause health problems, leading to kidney failure. Barium (Ba) is found in some household products and, although these products represent a small part of Ba consumption, they have large toxicological significance. Elements like Antimony (Sb) and Uranium (U), which are not abundant in nature, deserve special attention, as they have toxicity in small doses (Buschinelli, 2020; Nordberg et al. 2014). Even though some of these elements are essential for human life, some of them, in excessive amounts, can cause health damage (Nordberg et al. 2014).

Since rural workers are exposed to pesticides and other xenobiotics like metals and metalloids in the same environment, research involving the synergistic action of this exposure has emerged. Some authors have already described synergic effects in the combinations of pesticides with other pesticides, with metals, and among several metals, resulting in greater toxicity for combined substances in comparison with single substances (Chen et al. 2013; He et al. 2015; Nordberg et al. 2014; Rehman et al. 2017; Singh et al. 2017; Xu et al. 2017). Ratner et al. (2014) describe that subjects occupationally exposed to metals and/or pesticides are more likely to have Parkinson's disease at early ages. Vinceti et al. (2012) found suggestive epidemiological evidence that Se and pesticides exposure are likely to be involved in amyotrophic lateral sclerosis etiology.

It is known that compounds, when mixed, can affect each other's toxicity, increasing or decreasing the resulting toxic effect, even showing cumulative effects. Individual toxic effects of pesticides and metals are well reported in the literature (Singh et al. 2017). In their study on carbofuran genotoxicity in human lymphocytes, Sharma and Sharma (2012) reported that this pesticide could cause oxidative stress by inducing the formation of free radicals. Besides, Sharma et al. (2014) and Singh et al. (2018), in their literature reviews, show that some heavy metals can indirectly induce to oxidative stress. The authors point out that this phenomenon can be related to the occurrence of diseases in humans, thus, emphasizing that both xenobiotics, despite presenting different toxic mechanisms, can lead to the same outcome.

Therefore, it is widely known that joint exposure to insecticides and metals poses a significant risk to human and animal health and the environment. Therefore, it is a topic that still needs to be better explored (Chen et al. 2013; He et al. 2015; Rehman et al. 2017; Xu et al. 2017). Moreover, as far as we know, no data have been found in the literature reporting the concentration of trace elements in biological samples from family farmers occupationally exposed to pesticides pointing to an association of these elements with exposure characteristics of the population.

Therefore, this study aimed to identify the association effect of trace elements in plasma and urinary excretion with pesticides used by a group of vineyard workers. Social information, work conditions, health conditions, alcohol consumption, and smoking habits were assessed during biological sampling involving vineyard workers of a northwest area of Paraná. The levels of 15 elements in plasma and urine were statistically evaluated to identify correlations and possible symptom causation effects derived from pesticide use.

Materials and methods

This is a cross-sectional study carried out in the rural region of Marialva, northwest of the state of Paraná (Brazil), in 2017. Marialva has approximately 35,800 inhabitants and it is a major grape producer, known as the Brazilian Capital of Fine Grapes (IBGE 2020). According to a 2018 report on gross production value, issued by the Brazilian Secretariat of Agriculture and Supply, grapes were among the main fruits produced in Paraná, representing 66% of its fruit production income. Marialva was responsible for 40% of the whole state's grape production, while other cities provided between 2 and 4% of the production (SEAB 2018).

Participants were intentionally selected in partnership with technicians from the Paraná Rural Development Institute (IDR) of Marialva, which provided the register of family farmers involved in grape cultivation. 36.8% of the 76 producers included in the IDR database refused to participate in the investigation due to lack of time to receive the research team. However, during the field research on rural properties, 18 grape producers (not registered in the IDR database) were asked to take part in the research, and were, then, included. Regarding the inclusion criteria adopted, producers had to be adults, of both sexes, family farming winegrowers, living in the rural area of the municipality that was the focus of the study. In addition, they had to accept the research participation terms on a voluntary basis. Considering workers and their family members who lived in the properties, 216 people over 18 years old participated in the study.

This study was approved by the Standing Committee on Ethics in Research Involving Human Beings (COPEP) of the State University of Maringá (opinion No. 2.068.991). In addition, all participants signed the consent form in order to participate in this research.

Data and biological material collection

We visited the farms twice to collect biological material and interview the participants. Data were collected through face-to-face interviews with each one of the farmers. The questionnaire was developed according to the Roadmap for Chronic Pesticide Poisoning Evaluation of the Paraná State Department of Health (SESA 2013), with information about gender, age, use of PPE, type of contact (occupational or environmental), exposure time, symptoms related to chronic exposure to fungicides, among others. This roadmap is a reference guide in Paraná for populations there are exposed to pesticides.

Regarding the symptoms associated with chronic exposure to fungicides, a list was made including fifteen neurological, dermatological, respiratory, and gastrointestinal symptoms, from the Roadmap for Chronic Pesticide Poisoning Evaluation (SESA 2013). The vineyard workers were asked if they had any of these symptoms at the time of the interview, and we calculated, for the PCA analysis, how many symptoms each respondent reported.

Biological material collection was performed at the farmers' workplace, as in other occupational exposure studies, taking the necessary precautions to avoid contamination. Concerning blood and urine sampling, collection was carried out before the beginning of the morning work to avoid contamination through working clothes. For urine sampling, workers were requested to wash their hands before collection. Blood samples were collected by conventional venous puncture and stored in a heparinized free of metals 6.0 mL vacuum collection VACUETTE® tubes. Urine samples were collected in metal-free flasks. This type of flask is commonly used for urine collection and posterior assessment of metals, as it does not unleash plastics to the urine, which could influence the metal analysis. After collection, both urine and blood samples were sent to the Toxicology Laboratory of the State University of Maringá, under refrigeration.

Subsequently, blood samples were centrifuged at 2000 rpm for 10 min to separate plasma from other blood elements. Since the samples would later be screened for metals in a different laboratory, plasma was preferred as a matrix over more commonly used whole blood, because long storage periods were unavoidable, and that could lead to variations in later metal analyses. Additionally, plasma also indicates the circulating metal content.

Both sample types (urine and plasma) were fractioned in three Eppendorf™ metal-free tubes at a volume of 200 μL, frozen at − 20 °C, and later sent to colleagues at the Center for Natural and Human Sciences, at the Federal University of ABC (Santo André, SP, Brazil) for metal analysis.

An enzymatic colorimetric method, from 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.

Metal analysis

Each plasma and urine sample was screened for Al, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Cd, Sb, Ba, Hg, Pb, and U with a technique for fast determination of metals in biological material using an inductively coupled plasma mass spectrometer (ICP-MS). The technique was previously validated and is described by Batista et al. (2009) and Batista et al. (2009). After homogenization, both urine and plasma samples were diluted 1:10 in a solution containing HNO3 0.5% v/v + Triton X-100 0.02% m/v. The samples were injected into an inductively coupled plasma mass spectrometer (ICP-MS Agilent 7900, Hachioji, Japan). Blanks and reference materials were run in each batch of analysis. All ICP-MS operational parameters are shown in Table 1.

Reference values for trace elements, in biological material of unexposed populations, are scarce in the literature. They are even rarer when it comes to metals in plasma. Therefore, the results of this study were compared with published research with reference values for the Brazilian population and values found for an unexposed population from the region of the population investigated by this study.

Statistics

Descriptive statistics were used for the interview results. The Kolmogorov-Smirnov normality test was used to verify if the variables likely followed a normal distribution. Principal component analysis (PCA) was used as an exploratory analysis method to identify associations between elemental content in biological samples and the information obtained from the interview. The list of variables included the elemental concentration of Al, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Cd, Sb, Ba, Hg, Pb, and U in plasma and urine; gender, age, educational level, type of contact with pesticides (occupational or environmental), exposure time, PPE use, health problems, symptoms related to chronic exposure to fungicides, alcohol consumption, and smoking. Principal components were considered significant for eigenvalues ≥1.0. Factor loadings which indicate the strength of each parameter's influence within a component

Table 1 Conditions and operational parameters for ICP-MS analysis

Monitored isotopes	
Helium mode (LoD in µg L^{-1})	^{27}Al (0.012), ^{52}Cr (0.005), ^{55}Mn (0.004), ^{59}Co (0.0001), ^{60}Ni (0.02), ^{63}Cu (0.015), ^{66}Zn (0.01), ^{75}As (0.003), ^{82}Se (0.24), ^{111}Cd (0.004), ^{121}Sb (0.0006), ^{138}Ba (0.002), ^{202}Hg (0.00005), ^{208}Pb (0.007), and ^{238}U (0.0002)
Internal standard	^{89}Y 10 µg L^{-1}
Peak pattern / replicates /Ssweeps	3 / 2 / 100
Radio frequency power	1550 W
Argon flow rate	15 L min^{-1}
Sample uptake / speed / stabilize	10 s / 0.3 rps / 6 s
Nebulizer pump (acquisition)	0.3 rps
Carrier nebulizer gas flow rate	1.05 L min^{-1}
Nebulizer type	Mira Mist™
Spray chamber	UHMI Quartz Spray Chamber
Temperature	2°C
Torch (2.5 mm) sample depth	6 mm
Interface	Nickel cones
Sampler cone	1.0 mm
Skimmer	0.9 mm
Collision cell	Helium > 99.999 %

LoD, limit of detection; *rps*, rotation per second

were considered for values ≥0.6. PCA was the main statistical test applied in this study because of its ability in identifying the variables that affect and explain most of the system variability. On one hand, as a normalized statistical technique, PCA does not struggle with confounding variables as other statistical tests do. On the other hand, confounders are part of the information vector composed by measured variables. Thus, information like age, sex, and alcohol consumption can explain the system variability by enhancing or hindering the measurands signal. By the test operation principle, the PCA plot shows any confounder effect that is included in the variability matrix.

Results

Characteristics of the studied group

This study was carried out with an intentional sample consisting of 216 family farmers involved in grape cultivation. There were 123 (56.9%) men and 93 (43.0%) women, with an average age of 47.1 years. Table 2 presents the information obtained from the interviews with the population investigated. Their education level ranged from no education to higher education (more than 12 years). Most participants (47.7%) had on average 8 years of study. Regarding health, 51.8% did not report any health problem at the time of the interview. Almost half (44.9%) reported alcohol consumption and a few (8.8%) reported being smokers.

Concerning work conditions, 79.63% of the population reported, that they were exposed to pesticides because of work. The main forms of contact were related to the preparation, application, transport, and storage of pesticides, and during cleaning and maintenance of the equipment used in their application. The average exposure time of the population investigated in this study was of 21.9 years. The exposure time of 28.2% of them was of 11 to 20 years, whereas 23.6% of the farmers were exposed to pesticides for 21 to 30 years. Just a few workers (n = 13; 6.02%) stated to have used all the recommended PPE (overalls, gloves, boots, visor, masks) during contact with pesticides and 105 (48.61%) declared they made partial use of PPE. As a matter of fact, 22 vineyard farmers reported having been intoxicated by pesticides acutely, and 10 of them sought medical care.

Use of pesticides

Regarding the use of chemical substances, we made a list of the substances recommended for the cultivation of grapes by the IDR-Marialva-PR. During the interview, the workers answered whether they used these substances or not. All active ingredients belong to the fungicide class, except for cyanamide, that is a growth regulator. Frequency of use in the grape field by farmers and the toxicological classification are shown in Table 3. That list corresponds to the most recent exposure. However, for most of the long-term exposed subjects, it is difficult to have the full list of substances used since the moment they started dealing with them.

Table 2 Characteristics of the vineyard farmers who took part in this study

Variable	n (%)	Mean±SD
Age (years)		47.10 ± 13.07
Gender		
Men	123 (56.94%)	
Women	93 (43.06%)	
Educational level (years)		8.72 ± 2.15
None	4 (1.85%)	
≤8	103 (47.69%)	
9–11	74 (34.26%)	
≥12	3 (1.39%)	
Reported any health problem		
Yes	78 (36.11%)	
No	112 (51.85%)	
Alcohol consumption		
Yes	97 (44.91%)	
No	92 (42.13%)	
Smoking habits		
Smokers	19 (8.80%)	
Non-smoking	170 (78.70%)	
Contact type		
Environmental	16 (7.41%)	
Occupational	172 (79.63%)	
Exposure time (years)		21.89 ± 12.46
≤1–10	38 (17.59%)	
11–20	61 (28.24%)	
21–30	51 (23.61%)	
31–40	22 (10.19%)	
>40	14 (6.48%)	
Use of recommended PPE		
All	13 (6.02%)	
None	72 (33.33%)	
Partially	105 (48.61%)	
Previous acute intoxication		
Yes	22 (10.19%)	
No	168 (77.78%)	

PPE, personal protective equipment

Outliers

Twenty-three individuals with elemental concentration were considered outliers in relation to the studied group. These individuals corresponded to 11 men and 12 women, aged 22 to 65 years (48.9 years on average). Most of them were occupationally exposed to pesticides (78.3%), with an average exposure time of 26.4 years (ranging from 1 to 57 years), and only one out of 23 workers reported the use of all PPE components. The values were considered outliers simply because the concentration values were larger than the group average plus 3 x SD (see Online Resource 1).

Subjects with normally distributed values

The results obtained for each element quantified in plasma and urine were presented as mean (minimum-maximum), as the data showed normal distribution. Table 4 presents the results obtained for the vineyard farmers' urine compared to a population not exposed to pesticides and metal (Rocha et al. 2016) and with the results found by Batista et al. (2009), that present reference concentrations for trace elements in urine for the Brazilian population. In order to compare data results showing different distributions, results in Table 4 are presented as central tendency measures.

In comparison with the Brazilian reference values (Batista et al. 2009), Al, Mn, Co, Ni, Cu, Se, Ba, and Pb of the exposed ones were above the reference value established for an unexposed population. Comparing with the values of the study carried out in Maringá, Cr, Mn, Co, Ni, and Zn increased for both genders of the exposed population. Cu and As had a lower mean in exposed individuals. However, the maximum values found were greater than the confidence interval presented by Rocha et al. (2016).

Plasma elemental content is shown in Table 5, with the values obtained by Rocha et al. (2016), which were used for comparison. Both men and women had Cr and As reduced values in comparison to values from Rocha et al. (2016). In contrast, an increase of Mn and Zn was observed in this study's population, in both genders, in comparison to the unexposed population (Rocha et al. 2016). The Cu average was higher for men in the exposed population and lower for women. However, women from the exposed population had higher maximum values. For Co and Ni, there was no difference between the population means. Nonetheless, the maximum values found for the population of our study were higher than those of non-exposed populations.

Principal component analysis

For the PCA sample matrix, the number of subjects considered for the study was 375 (216 individuals in two collection moments), with 35 variables (see Online Resource 2). A total of 319 cases were considered valid for PCA assessment. Principal component analysis identified six main principal factors that were able to explain 41% of the system variability.

Factor 1 explained 12.44% of the system variability. The main cluster of variables linked with factor 1 was composed of the elemental content in urine. In this cluster, we found the urine content of Al, Sb, Pb, and Se with a strong (loading <− 0.7) inverse correlation associated with the urine content of U, Cu, Zn, Mn, Ba, and Ni, also with a moderate inverse correlation (loading <− 0.5), as presented in Fig. 1 (Cluster 1). The positive correlations under factor 1 were weak (loading > 0.1) for alcohol consumption, EPI use, the plasma content of Sb,

Table 3 Percentage of use and toxicological classification of pesticides used in vineyard farmers' properties

Active ingredients (commercial name)	Toxicological class	%
Cyanamide (Dormex®)	III	95.8%
Methyl thiophanate (Cercobin®)	V	95.8%
Propiconazole + Difenoconazole (Score Flexi®)	V	95.3%
Pyraclostrobin + Metiram (Cabrio Top®)	IV	95.3%
Mancozebe (Dithane®)	V	93.7%
Copper oxychloride (Recop®)	V	92.1%
Benalaxil + Mancozeb (Galben-M®)	V	88.9%
Cymoxanil + Mancozebe (Curzate®)	V	88.4%
Fosetyl (Aliette®)	Not classified	83.7%
Fenamidone (Censor®)	V	77.9%
Metalaxyl-M + Chlorotanil (Ridomil Gold Bravo®)	III	72.1%
Folpete (Folpan®)	V	70.0%
Tebuconazole (Folicur®)	V	66.3%
Iprodiona (Rovral®)	V	65.3%
Mancozebe (Manzate®)	V	59.5%
Copper hydroxide (Garant®)	V	46.8%
Azoxystrobin + Diphenoconazole (Amistar Top®)	V	36.3%
Captana (Captan®)	IV	26.3%

[a] Toxicological class was according on ANVISA (2015). III: moderately toxic; IV: slightly toxic; V: unlikely to cause acute damage; Not classified: product not classified as toxic

and education. However, these variables were not considered statistically significant.

Factor 2 explained 7.8% of the system variability and had a moderate to a weak inverse correlation that associated the plasma content of Se, Zn, Ba, Mn, Cr, Ni, Al, Pb, and Cu (Loading < −0.4) with the positive weak correlation of urine and plasma As content, age, and smoking (see Cluster 2, Fig. 1).

Factor 3 has two groups of variables that correlate inversely. Exposure time and age with plasma content of Al, Cr, and Ni are associated with each other, with loading <−0.4. Those were inversely correlated to an increase in education level, alcohol consumption, and urine content of Pb, U, and Sb (loading >0.2), as shown in Fig. 1, Cluster 3.

Regarding these cases, a single variable or an individual cluster was not able to explain the increase of symptoms, nor any of the isolated factors. It was noticed that a vector in the second quadrant of Factor 1 and Factor 2 (-aF1, +bF2), or the third quadrant of Factor 1 and Factor 3 (-aF1, -cF3), correlates with the increase of the sum of symptoms. It means that the increase in the number of symptoms is somehow associated with the increase of urinary Al, Sb, Pb, Se, U, Cu, Zn, Mn, Ba, and Ni content (-F1), with age, uAs and pAs (+F2), and with the decrease of plasmatic concentration of Se, Zn, Ba, Mn, Cr, Ni, Al, Pb, and Cu (-F2). The sum of symptoms also correlated with an increase in exposure time, age, and plasma content of Al, Cr, and Ni (-F3), and with a decrease in educational level, alcohol consumption, and urinary levels of Pb, U, and Sb.

As presented in Fig. 1, the PCA shows that an increase in the number of symptoms is associated with the increase of plasmatic and urinary As, while the increase in urinary excretion of Al, Ba, Cu, Mn, Ni, Pb, Sb, Se, Zn, and U also is associated with the increase in the number of symptoms.

Discussion

Farmers are occupationally exposed to multiple pesticides and environmentally subjected to trace elements through food, drinking water, air, and smoking (Flora 2016; Jayasumana et al. 2015). It is already known that the application of Cu-based pesticides is the main form of contamination by Cu in grapes (Li et al. 2018). Moreover, some pesticides contain metals in their formulation, and studies report that it could be synergistic effects of pesticides and metals (Flora 2016; He et al. 2015; Singh et al. 2017).

Traditional studies are frequently performed with a single pesticide, defining individual reference doses or occupational exposure limits for each product (Alves et al. 2016). Nonetheless, in day-to-day practice, exposure happens to many compounds simultaneously (Kennedy et al. 2015; Tsatsakis et al. 2016).

Human exposure to trace elements found in pesticide formulations can occur through several routes, especially the respiratory one, mainly when there are difficulties regarding the use of protective equipment. Exposure can happen in transportation,

Table 4 Comparison between trace elements levels in urine for winegrowers (this study) and non-exposed populations (Rocha et al. 2016 and Batista et al. 2009)

Element	This study (μg g^{-1} creat)			Rocha et al. 2016 (μg g^{-1} creat)		Batista et al. 2009 (μg g^{-1} creat)
	Male [a]	Female [a]	Both genders [a]	Male [b]	Female [b]	Both genders [c]
Al	26.58 (<LOQ–145.29)	35.46 (<LOQ–222.19)	30.2 (<LOQ–222.2)	-	-	3.4 (0.22–17.5)
Cr	1.07 (<LOQ–18.87)	2.53 (<LOQ–68.45)	1.5 (<LOQ–45.2)	2.22 (1.97–2.49)	1.99 (1.60–2.47)	-
Mn	1.19 (<LOQ–18.77)	2.39 (<LOQ–29.37)	1.7 (<LOQ–29.4)	0.93 (0.85–1.03)	1.10 (0.96–1.27)	0.90 (0.50–4.40)
Co	0.48 (<LOQ–3.33)	0.83 (<LOQ–7.38)	0.6 (<LOQ–7.4)	0.20 (0.18–0.23)	0.25 (0.20–0.31)	0.20 (0.32–3.7)
Ni	2.32 (<LOQ–31.56)	4.45 (<LOQ–71.02)	3.2 (<LOQ–71.0)	1.52 (1.29–1.79)	1.48 (1.05, 2.07)	1.30 (0.10–4.20)
Cu	14.16 (<LOQ–305.95)	23.74 (<LOQ–577.70)	14.3 (<LOQ–111.4)	24.53 (22.80–26.39)	27.01 (24.13–30.23)	0.90 (2.20–18.40)
Zn	409.77 (<LOQ–1839.50)	421.14 (<LOQ–1854.66)	412.7 (<LOQ–1854.7)	228.90 (207.10–253.00)	186.90 (154.90–225.50)	-
As	9.70 (<LOQ–173.40)	8.13 (<LOQ–69.77)	8.3 (<LOQ–69.8)	12.48 (11.39–13.67)	11.34 (9.72–13.23)	-
Se	17.65 (<LOQ–70.22)	27.78 (<LOQ–713.17)	19.7 (<LOQ–122.3)	-	-	2.80 (5.20–98.90)
Cd	0.26 (<LOQ–5.60)	0.38 (<LOQ–8.16)	0.3 (<LOQ–8.2)	-	-	0.30 (0.05–0.83)
Sb	0.08 (<LOQ–0.68)	0.09 (<LOQ–0.60)	0.1 (<LOQ–0.7)	-	-	0.20 (0.03–2.14)
Ba	4.13 (<LOQ–29.63)	5.51 (<LOQ–21.07)	4.7 (<LOQ–29.6)	-	-	1.50 (0.20–5.30)
Hg	0.12 (<LOQ–2.43)	0.11 (<LOQ–1.04)	0.1 (<LOQ–2.4)	-	-	-
Pb	1.26 (<LOQ–4.90)	1.76 (<LOQ–33.29)	1.3 (<LOQ–7.3)	-	-	0.80 (< 0.03–2.96)
U	0.004 (<LOQ–0.022)	0.01 (<LOQ–0.131)	0.004 (<LOQ–0.032)	-	-	0.008 (0.003–0.056)

[a] Values are expressed in mean (minimum–maximum)

[b] Values are expressed in geometric mean (95% CI GM)

[c] Values are expressed in mean (5th–95th percentile)

Table 5 Comparison between trace elements levels in plasma for winegrowers (this study) and non-exposed populations (Rocha et al. 2016)

Plasma	This study ($\mu g\ L^{-1}$)		Rocha et al. 2016 ($\mu g\ L^{-1}$)	
	Male [a]	Female [a]	Male [b]	Female [b]
Al	22.43 (<LOQ–46.44)	22.30 (9.60–47.41)	-	-
Cr	0.29 (<LOQ–1.89)	0.28 (0.07–1.74)	1.95 (1.87–2.04)	1.96 (1.83–2.11)
Mn	0.95 (<LOQ–5.29)	1.00 (0.39–4.50)	0.53 (0.48–0.59)	0.56 (0.49–0.64)
Co	0.14 (<LOQ–0.72)	0.18 (0.05–1.34)	0.15 (0.14–0.16)	0.16 (0.13–0.19)
Ni	0.73 (<LOQ–11.70)	0.91 (<LOQ–16.03)	0.70 (0.61–0.80)	0.84 (0.67–1.05)
Cu	1106.66 (41.63–1999.27)	1371.81 (569.23–2605.50)	891.40 (855.50–928.80)	1401.00 (1270.00–1545.00)
Zn	1003.39 (25.31–1900.23)	961.98 (463.94–1436.74)	738.90 (708.50–770.70)	700.90 (649.10–756.70)
As	0.24 (<LOQ–1.44)	0.14 (<LOQ–1.07)	1.15 (1.09–1.21)	1.20 (1.10–1 .30)
Se	86.99 (1.46–180.64)	87.42 (33.51–172.26)	-	-
Cd	0.30 (<LOQ–11.38)	0.47 (<LOQ–9.43)	-	-
Sb	3.79 (0.15–9.65)	3.52 (1.74–8.76)	-	-
Ba	1.08 (<LOQ–4.34)	1.17 (0.05–5.33)	-	-
Hg	0.26 (0.03–2.03)	0.24 (0.06–1.67)	-	-
Pb	0.81 (0.12–7.14)	0.82 (0.30–10.03)	-	-
U	0.003 (0.001–0.012)	0.003 (0.001–0.020)	-	-

[a] Values are expressed in mean (minimum–maximum)

[b] Values are expressed in geometric mean (95% CI GM)

handling for storage, preparation, application, and washing of containers and clothes used at work. Those are situations in which these elements, in the form of fine particles, can reach the human respiratory system, causing local effects, such as mucosal irritation, but also systemic effects after absorption. Dermal and ocular exposure is also common during the aforementioned tasks, when handling can cause local (irritation, dermatitis, and other lesions) and systemic effects. Oral exposure also occurs, since hygiene measures tend to be precarious. Water and food are consumed during work without the removal of contaminated clothing, not to mention that workers tend to not wash their face and hands. In addition to these types of exposure, there is also contamination through water, food, and air, often because farmers' houses are usually near the crops (ATSDR 2019; Yarpuz-Bozdogan 2018; López-Gálvez et al. 2019; Nordberg et al. 2014).

Because of the complexity of the subject, this study will not go deeper into discussing the toxic effects of each element, but rather contribute with values of trace elements in biological material of vineyard farmers who have chronic exposure to pesticides. In addition, it intends to demonstrate an association between the characteristics of fungicide use and trace elements concentration in biological material.

The results demonstrate that this population of vineyard farmers has been recently exposed to pesticides that belong mainly to the fungicide class, and to unknown pesticides for over 20 years on average. Furthermore, cases of acute poisoning involving pesticides were reported, and only a few farmers said that they used the recommended PPE for handling and applying pesticides. This demonstrates that these workers face an aggravating factor in pesticide exposure, since they do not rely on the use of protective equipment. Unfortunately, these findings corroborate other Brazilian studies that report the lack of use of this equipment by rural workers (Sousa et al. 2016; Viana et al. 2017).

The literature is scarce in terms of a reference value for trace elements in biological material, due to variations among regions (Batista et al. 2009). One of the limitations of our research was the impossibility of using a control group, since the region in which the study was carried out is mostly rural, which makes it difficult to find people not exposed to pesticides to compose the control group. Because of this variation among regions, we chose not to compare the results of this study with foreign literature. Therefore, values found in other studies from Brazil (Batista et al. 2009) and Paraná (Rocha et al. 2016) were used to compare the values of trace elements found for vineyard farmers. Regarding plasma analysis, it was not possible to compare all trace elements levels with other references, as whole blood was the matrix of choice in the other studies.

As for pesticides, this study reports only the substances recommended by the IDR-Marialva-PR for the cultivation of grapes, which were the ones recently used by the population in question. It is important to highlight that farmers may have

Fig. 1 Principal component analysis with Factor 1 versus Factor 2 and Factor 1 versus Factor 3 loading plots by elements (above); Factor 1 versus Factor 2 and Factor 1 versus Factor 3 loading plots by individuals (below). Factor 4 (blue) and Sum of symptoms (orange)

been exposed to other pesticides in the past, and long-term exposure may be related to the current health condition (Carneiro 2015; SESA 2013). Due to recent use of pesticides, fungicides containing the active ingredient metiram have Zn in their formulation. The ones that contain mancozeb have Mn and Zn. Others with Copper oxychloride and Copper hydroxide have Cu as their base formula (ADAPAR 2020). Studies report other elements that can be present in pesticide formulation, such as As, Cd, Cr, Pb, and Ni (Cestonaro et al. 2020), and the vineyard workers were exposed environmentally to a variety of pesticides, since the rural region has other crops besides grape. It is worth mentioning that farmers showed higher values of Cu, Ni, Mn, and Zn in plasma and/or urine, when compared to at least one of the studies involving unexposed populations.

Moreover, men are more often involved in handling pesticides than women, and the results showed that men had a higher amount of Cu in plasma. In addition, the fact that men have a higher amount of Cu in plasma and not in urine may indicate accumulation of this element in their body. As for As, Cd, Cr, and Pb, although the average was lower, the maximum values for the exposed population were higher, thus, evidencing that there are farmers with high value for these elements, even in cases in which the average was low.

The absence of PPE, associated with the results of trace elements in biological material, and the presence of these same elements in the fungicide used by this population may suggest that vineyard workers are exposed to metals by fungicides. Other studies, such as that by Mora et al. (2014), reported that women exposed to mancozeb may have higher blood and hair Mn concentrations when compared to non-exposed women. Thompson et al. (2012) reported that Cu (from Copper-based fungicidal) in buccal cells of vineyard workers increases when there is also an increase in contact with fungicides. Other studies also support the fact that pesticides can be sources of exposure to metals (Ghazali et al. 2012; Gunier et al. 2013).

Regarding trace elements in urine, Brazilian legislation presents Biological Indicators of Excessive Exposure for As (35 µg/g creatinine) and Cr (25 µg/L), and Biological Exposure Indicators with Clinical Significance for Cd (5 µg/g creatinine) (MTE 2020). These indicators refer to occupational exposure to metals. Although the studied population is not considered occupationally exposed to these elements, the urine of eight vineyard farmers showed higher values of As, Cd, and Cr than those recommended by the Brazilian Regulatory Standard 7 (MTE 2020). This emphasizes the fact that this vineyard workers population was exposed to these elements possibly due to contact with pesticides (Chen et al. 2013). Furthermore, studies suggest that human co-exposure to Cd and As can cause more pronounced renal damage than exposure to each element alone (Nordberg 2010). Due to the examination results, these workers were referred to the health service, so that a medical team could assist them.

It is complex to analyze the concentration of trace elements in biological material, mainly when it comes to plasma and urinary concentration of these elements. We hope that, by analyzing the elements in plasma and urine, this study will contribute with filling in the literature gap regarding values of trace elements in both biological materials for workers exposed to pesticides. It is important to highlight that plasma concentration indicates recent exposure and accumulation in body organs. As for urine concentration, it is a biomarker commonly used for trace elements' body burden or kidney levels (Nordberg 2010).

Principal component analysis has been used in other studies as an exploratory data analysis tool, as it allows the identification of possible correlations between variables (Freire et al. 2020). In this study, which made use of PCA analysis, it was possible to note the synergism and antagonism between different elements in human biological material and other variables, as shown in Fig. 1. The closer the points, the greater the synergistic effect, and the more distant the points, the greater is the antagonistic effect. Points that are in different quadrants, such as education and exposure time (cluster 3), vary in an antagonistic way, so the higher the education level, the shorter the exposure time. This is also true for the elements in plasma and urine, in which the greater the amount of the element in urine (greater elimination), the less the amount in the plasma.

As already described in the literature, higher concentrations of some metals can interfere with the absorption and elimination of other metals (Singh et al. 2017). In addition, PCA analysis showed that alcohol consumption was related to the decrease in plasma levels of Al, Cr, and Ni. This must be due to the action of alcohol, which can increase the speed of elimination of these elements through urine (Cederbaum 2012).

It is important to mention that, in PCA analysis, there was more than one factor influencing the increase in the number of symptoms reported by vineyard farmers. The increase in metals Pb, Cu, Zn, Mn, and Ni (metals found in pesticides) in urine showed a positive relationship with the rise in the number of symptoms. These symptoms, used for PCA analysis, were described in a guide material for chronic poisoning by pesticides (SESA 2013).

In addition, the increase in time of exposure to pesticides, increase in age, and decrease in education level also showed a positive relationship with the increase in the number of symptoms. The data suggested a cumulative effect of pesticide mixtures, which corroborates other studies that report long-term exposure and low education as aggravating factors for chronic intoxication (Bulka et al. 2019; Sousa et al. 2016; Viana et al. 2017).

Although for some elements the concentrations do not exceed the safety levels determined by law, these legal limits can underestimate the real health risk. Simultaneous exposure to different chemical substances, as it occurs in crops, such as grape production conditions, can promote synergistic effects (Adamkovicova et al. 2014; Singh et al. 2017).

Exposure to these compounds can occur through multiple sources, pathways, and routes, including occupational exposure in application or production, and agricultural workers' "take-home" exposure, which affects their families (Wickerham et al. 2012). Metals and pesticides have the same routes of exposure (Satarug et al. 2003), and follow similar kinetics and mechanisms of action (Singh et al. 2017). Toxicity of individual metals is well investigated, but that of metal mixing, an environmental reality, and with other compounds, such as pesticides, is still relatively obscure.

Chronic exposure in daily fieldwork to the mixture of pesticides with possible synergistic or additive effects requires further studies (Nicolopoulou-Stamati et al. 2016; Singh et al. 2017). The exposure of vulnerable groups, such as family farmers, the absence of scientific studies capable of proving its harm, and the fact that there are countless possible mixtures highlight the complexity of the subject (Colosio et al. 2013; Hernández et al. 2013; Sexton 2012). The combination of compounds with diverse toxic effects may result in unknown health effects. The determining "safe" levels of exposure to a single pesticide, as it is currently done, underestimate the real health effects.

One of the limitations of this study is its non-probabilistic convenience sample. Its results reflect only the reality of the population and the region in which it was carried out (vineyard producers in Marialva-PR). Future studies with other sampling approaches may provide results that can be extrapolated to other regions and populations. Nevertheless, the contribution of this study must be highlighted as an exploratory research work that helps as a basis for generating hypotheses.

Despite these limitations, our findings highlight the need to consider mixtures of compounds in studies of health outcomes concerning environmental exposures, rather than focusing only on individual agents. Evidences of this are studies showing that mixtures of compounds can result in cumulative effects, which may have a greater effect than isolated compounds, regardless of the mode of action of the individual components (Jacobsen et al. 2010; Rider et al. 2010).

Conclusion

Some degree of simultaneous exposure was observed in the studied population. Even though the main exposure was the use of fungicides, changes in the levels of Al, Cr, Co, Ni, Se, and Pb elements were detected in this research.

This work discusses the need of a new focus on agriculture procedures, seeking to reinforce the safe use and reduction of the number and quantity of chemical pesticides used. Although the risks of exposure to pesticides have been widely discussed in the current available literature, this study highlights recent findings of trace elements in biological material and their association with characteristics of pesticide use by a vineyard farmer's population that was simultaneously exposed to various chemical pesticides. Undoubtedly, further research needs to focus on this subject in order to demonstrate causality and elucidate mechanistic actions of pesticide mixtures.

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Author contribution All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by RSL, RGA, DH, SC, and SBN. Investigation and methodology were performed by RSL, FPP, TP, and BLB. Data analysis was performed by RSL and LRM. Funding acquisition was performed by MLFO, MMJ, and SAGM. Data curation, project administration, resources, and supervision were performed by MLFO and SAGM. The first draft of the manuscript was written by RSL and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Approval was obtained from the ethics committee of the State University of Maringa. The procedures used in this study are in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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