Research, Society and Development, v. 14, n. 2, e6214248248, 2025
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v14i2.48248

Study of trace organic contaminants (TrOcs) on the medicinal leaves of *Eugenia uniflora* L. from urban and forest-preserved areas

Estudo dos contaminantes orgânicos de traço (TrOcs) nas folhas medicinais de *Eugenia uniflora L.* de áreas urbanas e preservadas

Estudio de los contaminantes orgánicos traza (TrOcs) en las hojas medicinales de *Eugenia uniflora* L. de áreas urbanas y preservadas

Received: 02/07/2025 | Revised: 02/13/2025 | Accepted: 02/13/2025 | Published: 02/18/2025

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Abstract
The organic pollutants promote significant risks to both human health and environmental sustainability. These compounds have seen a dramatic increase in diversity and quantity, infiltrating bodies of water, marine environments, and living organisms. Some factors contribute to pollutant emissions, such as road traffic and industrial activities and it leads to a spatiotemporal variability. Between the organic pollutants, Trace Organic Contaminants (TrOCs), which comprise a diverse range of industrial chemicals, pharmaceutical residues, and pesticides, are also gaining attention for their toxic effects on aquatic organisms and plants. Thus, this investigation aimed to evaluate the occurrence of TrOCs deposited in *Eugenia uniflora* leaves from urban areas and preserved forests during the four seasons of the year. Experimental, laboratory research, of a quantitative nature, was carried out. The identification of emerging trace organic contaminants was analyzed by Gas Chromatography - Mass Spectrometry (GC-MS) and Gas chromatography - Flame Ionization Detector (GC-FID). This analysis allowed us to identify TrOCs and some of them related to endocrine disorders. These TrOCs differed depending on the region and season of the year in which the leaves were collected, and they were present even in plants cultivated in preserved forests.
Keywords: CG-MS; Organic pollutants; Trace organic contaminants; *Eugenia uniflora*.

Resumo
Os poluentes orgânicos representam riscos significativos tanto para a saúde humana quanto para a sustentabilidade ambiental. Esses compostos têm aumentado drasticamente em diversidade e quantidade, infiltrando-se em corpos d'água, ambientes marinhos e organismos vivos. Diversos fatores contribuem para a emissão desses poluentes, como o tráfego rodoviário e as atividades industriais, resultando em variabilidade espaço-temporal. Dentre os poluentes orgânicos, os Contaminantes Orgânicos de Traço (TrOCs), que incluem uma ampla variedade de produtos químicos industriais, resíduos farmacêuticos e pesticidas, vêm ganhando atenção devido aos seus efeitos tóxicos em organismos aquáticos e plantas. Assim, esta investigação teve como objetivo avaliar a ocorrência de TrOCs depositados em folhas

de *Eugenia uniflora* provenientes de áreas urbanas e florestas preservadas ao longo das quatro estações do ano. Uma pesquisa laboratorial, de caráter experimental e quantitativo foi performada. A identificação de contaminantes orgânicos emergentes foi realizada por Cromatografia Gasosa - Espectrometria de Massas (GC-MS) e Cromatografia Gasosa - Detector de Ionização por Chama (GC-FID). Essa análise permitiu a identificação de TrOCs, alguns dos quais estão relacionados a distúrbios endócrinos. Esses contaminantes variaram de acordo com a região e a estação do ano em que as folhas foram coletadas, estando presentes mesmo em plantas cultivadas em florestas preservadas.
Palavras-chave: CG-MS; Poluentes orgânicos; Contaminantes orgânicos de traço; *Eugenia uniflora*.

Resumen
Los contaminantes orgánicos representan riesgos significativos tanto para la salud humana como para la sostenibilidad ambiental. Estos compuestos han aumentado drásticamente en diversidad y cantidad, infiltrándose en cuerpos de agua, ambientes marinos y organismos vivos. Diversos factores contribuyen a la emisión de estos contaminantes, como el tráfico vehicular y las actividades industriales, lo que resulta en una variabilidad espacio-temporal. Entre los contaminantes orgánicos, los Contaminantes Orgánicos Traza (TrOCs), que incluyen una amplia variedad de productos químicos industriales, residuos farmacéuticos y pesticidas, han ganado atención debido a sus efectos tóxicos en organismos acuáticos y plantas. Así, esta investigaciín tuvo como objetivo evaluar la presencia de TrOCs depositados en hojas de *Eugenia uniflora* procedentes de áreas urbanas y bosques preservados durante las cuatro estaciones del año. Se llevó a cabo una investigación de laboratorio, de carácter experimental y cuantitativa. La identificación de contaminantes orgánicos emergentes se realizó mediante GC-MS y GC-FID. Este análisis permitió identificar TrOCs, algunos de los cuales están relacionados con trastornos endocrinos. Estos contaminantes variaron según la región y la estación del año en la que se recolectaron las hojas, estando presentes incluso en plantas cultivadas en bosques preservados.
Palabras clave: CG-MS; Contaminantes orgánicos; Contaminantes orgánicos traza; *Eugenia uniflora*.

1. Introduction

Organic pollutants are compounds that can be emitted intentionally or not, for example, by the consequences of urbanization and industrial processes, and have serious risks to human health (Markiewicz et al., 2017). In recent decades, there has been a drastic increase in the chemical variety and quantity of organic contaminants in bodies of water, marine environments, and living organisms, such as polychlorinated biphenyls, organochlorine pesticides, pharmaceutical products, and plastics (Sousa et al., 2017; Allan et al., 2006).

A lot of factors can modulate the emission of these contaminants. As they are emitted by road traffic and as industrial byproducts, the emission can vary by seasonality, especially the VOCs (volatile organic compounds) (Beckers et al., 2018). Road traffic emission is one of the most important factors of urban contamination (Björklund, 2011). Even this emission may vary by the season, as the climate changes with it, with the presence of phthalate esters, alkanes, and alkyl phenols in the road dust ((Helmreich et al., 2010); Björklund, 2010). Industrial emissions can also cause the accumulation of organic contaminants, changing the concentration of which compound by season (Zhang et al., 2017). Thus, the variation of organic pollutant accumulation is spatiotemporal.

Furthermore, all these compounds are subjected to depositions, the wet deposition by rain or snowfall and the wet deposition by gravity and dispersion, moving these contaminants between the environment and leading them to some surfaces (Nežiková, 2019).

In addition, the disposal of plastics in the environment, such as those coming from urban disposal, can release organic compounds such as phthalates, organophosphate esters, and bisphenols, which are recognized as the most critical, as they have endocrine disrupting action, deleterious effects on humans and the environment, and can be found in marine environments at a level of μg/l in estuarine and coastal waters (Hermabessiere et al., 2017; Zhang et al., 2018).

Beyond the Organic Pollutants, Trace Organic Contaminants (TrOCs) are chemical compounds including industrial chemicals, steroid hormones, pharmaceutical and cosmetical residues, pesticides, phytoestrogens, and others (Hai et al. 2014, Alexander et al. 2012) that can be found, mostly, in urban and industrialized areas (Burant et al., 2012). These organic compounds are gaining notoriety because they can present acute and chronic toxicity on aquatic organisms or plants, causing biodiversity

loss and affecting human health for their adverse effects (Hai et al. 2014). According to the literature, abiotic factors can change a lot in plant organisms such as secondary metabolites production by *Eugenia uniflora*, and even in the contaminant content, the environment is directly associated with the rainfall index, exhibiting a higher concentration mainly in dry seasons (Gonçalves, 2021; Zhou et al., 2011).

In this sense, this investigation proposes evaluating the presence of these pollutants deposited in the *Eugenia uniflora* leaves, in different areas of cultivation and seasons of the year to monitor their behavior considering the spatiotemporal variation of organic pollutants emission and deposition.

2. Methodology

An experimental laboratory research, of a quantitative nature, was carried out (Pereira et al., 2018) using descriptive statistics with mean value, standard deviations, amplitude with maximum and minimum value (Shitsuka et al., 2014).

2.1 Chemicals

The dichloromethane solvent was purchased from Mallinckrodt Chemicals and a homologous series of C_8-C_{20} *n*-alkanes standards was purchased from Sigma-Aldrich (St. Louis, MO). The commercial activated carbon used was of mineral origin (minimum surface area of 1000 m^2/g and ash content < 6%), obtained from the company Fábrica Brasileira de Catalisadores Ltda - Filter Sorbius-400. It has applications in removing low-concentration contaminants in solid, liquid, and gaseous matrices. The pollutant biphenyl was purchased from SpecSol, at the concentration of 100mg/mL.

2.2 Sampling

The specimen Eu1 (voucher SPFE 711) was cultivated in the metropolitan city of São Paulo, close to the Ayrton Senna highway (geographical coordinates: Lat: 23º29'091" S, Long: 46º30'301" O and Alt: 734 m). The specimen Eu2 (voucher SPFE 713) was located in a preserved region in the Natural Park of Mogi Affonso de Melo (SP), geographical coordinates: Lat: 23º29'294" S, Long: 46º11'683" O and Alt: 867 m. These samples are 31672.11 m apart from each other. According to the Permanent Commission for the Creation and Expansion of Protected Areas- CNAP, the Itapeti mountain range, where the Park of Mogi Affonso de Melo is located, was implemented as a Mosaic of Protected Areas to increase the protection of the area's fauna and other biotic values. It is a closed park managed by the Department of Greenery and the Environment of the municipality of Mogi das Cruzes, with visits allowed and supervised only upon authorization from the agency.

On the same day, samples (approximately 9 g) of *Eugenia uniflora* (Eu) leaves were harvested from urban (Eu1) and forest-preserved areas (Eu2) and stored separately in black plastic bags. Each one of the collections was performed in triplicate during morning, afternoon, and evening periods. Harvesting was performed during the four seasons of the year 2018 (summer: February 6th Autumn: April 3th, winter: July 5^{th,} and spring: October 4th). The summer collection comes with the suffix (su), winter (w), spring (sp) and autumn (a), for example, EU2su is the summer urban sample.

The identification of the species was carried out by Prof. Leonardo Dias Meireles from the School of Arts, Science, and Humanities, University of São Paulo (EACH-USP). The specimens were deposited at the SPF Herbarium (EACH-USP).

2.3 Leaf contaminants extraction

In this study, immediately before harvesting, the leaves of *Eugenia uniflora* were covered with commercial activated carbon and carefully packaged to avoid contact between them and the loss of carbonaceous material. The adsorbent was in contact with the leaves for 14 days in a sealed container.

After the contact period, the charcoal adhered to the surface of the leaves was transferred, with the aid of a brush, to a beaker and mixed with 20 mL of dichloromethane. The extract was then filtered through filter paper and the solvent was concentrated to 2 mL in a rotary evaporator at 60°C. The resulting mixture was filtered through a 0.45 µm membrane (MF-Millipore) and stored in darkness at 4 ºC until GC-MS analysis.

A control sample was prepared to monitor any pollutants identified in the GC-MS analysis from solvent impurities and/or commercial charcoal. To prepare this sample, an amount of charcoal, like that obtained during brushing the leaves, but without contact with the leaves, was added to 20 mL of dichloromethane and subjected to the same process described above.

2.4 Chromatographic analysis

Chromatographic analyses of the samples were carried out on an Ultra Shimadzu Model QP 2010 mass spectrometer and a BPX5 (5%-Phenyl)-methylpolysiloxane) capillary chromatographic column (30 m x 0.25 mm x 0.25 mm). Three microliters were injected in splitless mode (1/25) at an injector temperature of 260 ºC. Carrier gas was helium (99.998 %) at a flow rate of 2.5 mL/min. The oven program started at 60 ºC for 1 min. The temperature was incremented at 3 ºC/min to reach 200 ºC, then immediately incremented at 10 ºC/min to 280 ºC and remaining for 1.67 min. Detection was performed in SCAN mode (40 to 550 m/z) and the data acquisition rate was 1.27 scans per second. Only compounds identified with a relative match above 700, on a scale from 0 to 1000, were considered. To calculate the retention index, a solution of the C_8-C_{22} homologous series was injected. The analysis parameters for *n*-alkanes were the same as those used for the samples. To obtain the relative percentages (%) by peak area normalization of the identified compounds, all samples were analyzed in gas chromatography with a flame ionization detector (GC-FID) using an Agilent GC 6850 system. GC-FID was performed using the same chromatographic conditions used in the GC-MS analysis. All compounds were identified using NIST107 and NIST21 libraries, comparing the mass spectra data and by the retention index calculation with a solution of C_8-C_{22} homologous series by the Van den Dool and Kratz equation.

2.4.1 Pollutants content estimation

The pollutant biphenyl was injected in the same chromatographic conditions above, in three concentrations: 50, 25 and 12.5 µg/mL. With the peak area data, a calibration curve was designed with the aim of linear regression. The equation obtained is y = 1703066.8x - 15398484 and the $R^2 = 0.974$

2.5 Target metabolomics, seasonal and local variations

Metabolomic data (peak area, protonated ion, retention time, and fragmentation) were generated by gas chromatography coupled to a mass spectrometer. In addition, the data was submitted to the MetaboAnalyst 5.0 platform (PANG et al., 2021) for multivariate statistical analysis, including partial least squares discriminant analysis (PLS-DA). Filtering was performed using the relative standard deviation (RSD), and normalization was performed by autoscaling.

The data of industries in each local was obtained from its City Halls by e-SIC, a digital platform for informations solicitations via the brazilian Access to Information Law (12.527/2018)

3. Results and Discussions

TrOCs identified in *E. uniflora* leaves

Comparison of the GC-MS spectra of the analyzed samples with data from the NIST107 and NIST21 libraries and the calculation of the retention index of the compounds with the standard of the homologous series C_8-C_{22} allowed the identification of thirty-one compounds (Table 1), whose are recognized as TrOCs (Al-Akid et al., 2001, Fries et al., 2004, Castillo et al., 2011;

Sanches-Silva et al., 2007; Conacher et al., 1986, Magdouli et al., 2013; Schieweck et al., 2020). Of these, 3,3-dimethyl hexane (**3**) and butylhydroxytoluene (**17**) were identified in leaves from both areas (urban and preserved forest) and the different periods/seasons of the year. The others showed a direct relationship with the region where the plants were grown and/or with the period/season of the year the leaves were collected.

Table 1 - TrOCs identified on the *E. uniflora* leaves.

Compound No.	Name of identified compounds	IR	IR_{lit}	Average Relative Abundance (%) (*n=3*)							
				Samples from urban area				Samples from the preserved area			
				Spring	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
1	hexadecane	268	272	ND	ND	ND	1.9±0.03	ND	ND	ND	ND
2	3,3-dimethyl pentane	558	660	ND	ND	ND	ND	1.6±0.04	ND	ND	ND
3	3,3-dimethyl hexane	740	744	6.3±0.02	4.1±0.03	1.5±0.02	ND	3.1±0.02	ND	4.0±0.03	5.6±0.02
4	3-ethyl-2-methyl-pentane	763	765	ND	ND	2.5±0.03	ND	ND	ND	ND	ND
5	2-methyl-3-hexanone	780	820	ND	ND	1.9±0.02	ND	ND	ND	ND	ND
6	3-methyl-4-heptanone	929	932	ND	ND	1.7±0.02	ND	ND	ND	ND	ND
7	2-methyl octane	860	865	ND	2.0±0.02	ND	ND	ND	ND	ND	ND
8	2-octanol	1004	1002	ND	3.7±0.02	ND	ND	ND	ND	ND	ND
9	5,7-dimethyl undecane	1196	1190	ND	3.2±0.02	ND	ND	ND	ND	ND	ND
10	2,4-dimethyl-undecane	1206	1208	ND	ND	15.4±0.04	ND	ND	ND	ND	ND
11	4,4-dimethyl undecane	1221	1215	2.3±0.02	ND	ND	ND	ND	ND	ND	ND
12	4,8-dimethyl undecane	1225	1217	9.0±0.02	ND	ND	ND	ND	ND	ND	ND
13	3-ethyl-3-methyl decane	1233	1229	ND	ND	6.0±0.02	ND	ND	ND	ND	ND
14	2,3-dimethyl undecane	1256	1251	ND	7.8	ND	ND	ND	ND	ND	ND

15	1-dodecanol	1474	1470	ND	23.2±0.01	ND	ND	ND	ND	ND	ND
16	2,6-di-*tert*-butyl-4-(hydroxymethylene)-2,5-cyclohexandien-1-one	1482	1478	ND	ND	ND	ND	32.3±0.01	ND	ND	ND
17	butylhydroxytoluene	1526	1520	40.4±0.01	40.0±0.01	ND	1.0 ±0.04	ND	ND	18.8±0.03	34.9±0.02
18	5-phenyl decane	1528	1533	ND	3.6 ±0.02	ND	ND	ND	ND	ND	ND
19	5-phenyl undecane	1639	1633	ND	3.6 ±0.01	ND	ND	ND	ND	ND	ND
20	4-phenyl undecane	1667	1660	ND	3.8 ±0.02	ND	ND	ND	ND	ND	ND
21	6-phenyl dodecane	1732	1727	ND	4.5±0.01	ND	ND	ND	ND	ND	ND
22	5-phenyl dodecane	1739	1732	ND	3.1±0.01	ND	ND	ND	ND	ND	ND
23	4-phenyl dodecane	1747	1743	ND	3.2±0.01	ND	ND	ND	ND	ND	ND
24	3-phenyl dodecane	1764	1758	ND	2.5±0.01	ND	ND	ND	ND	ND	ND
25	3,5-di-*tert*-butyl-4-hydroxybenzaldehyde	1782	1772	ND	2.8 ±0.02	30.2±0.03	ND	ND	ND	ND	ND
26	5-phenyl tridecane	1817	1821	ND	2.8±0.01	ND	ND	ND	ND	ND	ND
27	6-phenyl tridecane	1828	1824	ND	4.6±0.03	ND	ND	ND	ND	ND	ND
28	4-phenyl tridecane	1844	1838	ND	2.4±0.03	ND	ND	ND	ND	ND	ND
29	di isobutyl phthalate	1891	1897	ND	ND	ND	6.3±0.03	ND	ND	ND	ND
30	tributyl acetyl citrate	2246	2250	ND	ND	ND	40.0±0.01	ND	ND	ND	ND
31	bis(2-ethylhexyl) phthalate	2559	2550	ND	ND	ND	5.9±0.02	ND	ND	ND	ND
Average total relative abundance of identified compounds (%)[1]				65.7±0.02	52.9±0.03	62.8±0.03	59.5±0.03	37.0±0.02	74.0±0.02	22.8±0.03	40.5±0.02

Source: Authors

IR: retention index calculated;
IR (lit): retention index obtained from reports in the literature (Adams 2007);
ND: unidentified compounds in the related sample;
[1]values below 100% because the compounds eluted before the first hydrocarbon of the standard or after the last hydrocarbon of the standard was not considered.

The estimated pollutants content variates between 9.04 to 11.01 µg/mL. The lower content is by 2,3-dimethyl undecane in EU1su and the highest contents are obtained from butylhydroxytoluene, with 9.44 µg/mL, diisobutyl phthalate, with 9.35 µg/mL and tributyl acetylcitrate, with the highest concentration of 11.01 µg/mL. Comparing the relative abundance of components **3**, **17,** and **25** both in the urban area and in the preserved forest, **17** is the most present in the leaves during the seasons of the year. Exceptions were observed only in the period corresponding to the summer for the preserved forest (Eu2), in which TrOCs were not detected, and in the autumn for the urban area (Eu1), where **25** was the most abundant.

Since **17** is degraded to **25** in the natural environment (Fries et al., 2002), it is suggested that the climatic conditions during autumn may have contributed to the oxidation of the alkyl substituent of the butylhydroxytoluene forming the metabolite 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde.

The majority presence of **17** in samples from urban areas can be associated with its wide use as an additive in various industrial sectors (food, cosmetics, packaging, polymer industry, etc.) for about 70 years (Babich, 1982). Its presence in preserved forests can be associated with its occurrence as a natural product in some species (*Trichilia emetica* and *Vitex trifolia*) and with the various factors that govern the production, distribution, and transport of TrOCs in the environment (Shahidi, 2000; Babich, 1982; Usman et al., 2016; Wee et al., 2020; Sarmah et al., 2020).

About the TrOCs that were determined under specific or exclusive conditions, evaluating the seasonality, it was observed that the volatile organic compounds (VOCs) **2, 11, 12,** and **16** were uniquely identified in the spring period of the year.

The 1-dodecanol (**15**), 2-octanol (**8**), open-chain alkanes (**7, 9,** and **14**), and linear alkyl benzenes (**7**, **9, 14, 15,** and **18-24**, **26-28**) were identified exclusively in the harvest period corresponding to the summer. Substituted alkanes and ketones (**4-6, 10,** and **13**) were detected only in the autumn period and hexadecane (**1**), tributyl acetylcitrate (**30**), and phthalate esters (**29** and **31**) were identified specifically in the period corresponding to winter.

Several factors may be associated with this behavior of the identified pollutants. One of them refers to the physicochemical properties of these compounds, such as volatility and solubility in water, which may favor their transport or their permanence in the leaves under the different rainfall, temperature, and humidity conditions studied (Zalakeviciute et al., 2018; Rahman et al., 2022). Another factor that may be related to the presence or absence of these compounds at a given time/season of the year is the seasonality of their generation sources, such as between agricultural harvests and industrial production periods (Bodor et al., 2020; Chang et al., 2022).

This is likely because TrOCs are a large group of compounds emitted by both natural and anthropogenic sources (Duan et al., 2023). The presence of VOCs in the urban and rural areas has been reported in the literature and corroborates the results found in this study, showing that aliphatic hydrocarbons, such as **2**, **11, and 12**, are VOCs present in both urban and rural areas and those terpenes such as **16**, are VOCs dominant in the rural area (Roba et al., 2014; Adamová et al., 2020).

Of the four VOCs identified, 2,6-di-*tert*-butyl-4-(hydroxymethylene)-2,5-cyclohexandien-1-one (**16**) was the majority, corresponding to 32.3% of the peak abundance found by GC-MS in Eu2 leaf sample. The predominance of this compound in the preserved forest sample can be attributed to its classification as a terpene emitted from vegetation, specifically in rural forested areas (Roba et al., 2014; Adamová et al., 2020).

The spring TrOCs were the only present in the leaves of Eu1 and Eu2, as the other TrOCs from summer, autumn, and winter were identified only in the leaves of *E. uniflora* that grew in the urban area (Eu1). This relationship can be justified by the types of TrOcs identified in these samples that are exclusively generated by anthropic sources (transport sector, industrial activities, combustion processes, etc.).

Among the fifteen TrOcs found in summer, the most abundant was 1-dodecanol (**15**) (23.2% relative abundance). The compound 2-octanol (**8**) and thirteen linear alkyl benzenes (**7**, **9**, **14**, **18-24** and **26-28**) were also detected.

These long-chain alcohols (**8** and **15**) are used in various industrial processes, in the manufacture of surfactants, as flavor in the food industry and as an emollient in cosmetics, and also as a chemical intermediate for the production of various other chemicals (van Ginkel, 2007; Matos et al., 2019; Motteran et al., 2019). 1-dodecanol is also used as a thickening agent and raw material for surfactants and is one of the main VOCs emitted by the use of paints (Schieweck et al., 2015; National Center for Biotechnology Information, 2023).

The compounds identified in the autumn period (**4**-**6**, **10,** and **13**) were those of the class of substituted alkanes and ketones, with undecane, 2,4-dimethyl (**10**) being the one with the highest abundance (15.4 %).

The aldehydes/ketones are pollutants of the VOC class and are already reported as predominant in urban areas due to their wide use in industrial processes, especially plastics, and polymers, and as gasoline additives (Fenske, 1993; Hamadi, 2010; Almanzalawy et al., 2023).

Regarding the period corresponding to winter, four TrCOs were identified (**1**, **29**-**31**), including hexadecane; two substituted phthalates, and the majority compound tributyl acetylcitrate (**30**). The predominance of **30** over the others is probably related to its application in a greater number of sectors and industrial products (plastics, repellents, fibers, lubricants, packaging, pharmaceuticals, cosmetics, personal care products, automobiles, and civil construction) (Pandit et al., 2018; Horie et al., 2022; García-Pimentel et al., 2023).

Hexadecane is one of the main chemical compounds in smoke condensates from cooking oil and oil and gas industry activities (Morais et al., 2021; Szewczyńska et al., 2020). Bis (2-ethylhexyl) phthalate (**31**) is considered an important pollutant in the world released by industrial products (Magdouli et al., 2013). Tributyl acetylcitrate (**30**) is one of the compounds most detected in published studies of plastic pollutants (García-Pimentel et al., 2023).

Effect of spatiotemporal variation in *E. uniflora* individuals

As the TrOCs can be emitted by industrial activities (Burant *et al.*, 2018), the industries, as their sector in the vicinity of the individuals were evaluated, seeing that the organic compounds emitted are directly connected to the industrial activity. A study was therefore carried out to list the industries located within a 5 km radius of each individual studied. Of the eighty-one industries near Eu1, twenty six operate in the polymer, paint, adhesive, glass, and chemical sectors (Table 2), and their names are changed to not expose these establishments. On the other hand, the conditions in Eu2's neighborhood for the same distance differ, as there are only residences.

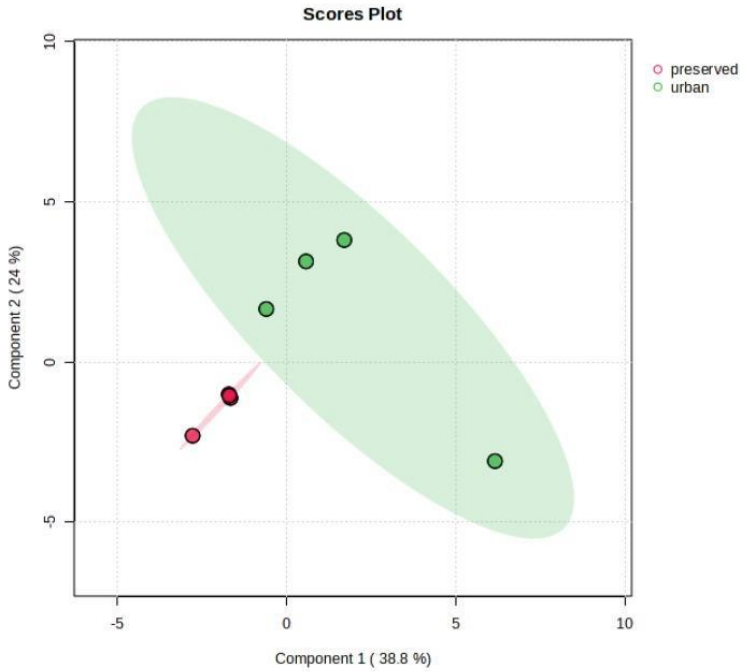
Table 2 - Industries and areas of activity in the vicinity of the individual 1

Industries	Sector	Distance to EU1
Industry 1	Tinkering	453.37m
Industry 2	Packaging	720.38m
Industry 3	Glassware	790.13m
Industry 4	Carpentry	1.11km
Industry 5	Carpentry	1.05km
Industry 6	Chemistry industry	1.71km
Industry 7	Polymers	2.05km
Industry 8	Polymers	5.46km
Industry 9	Packaging	2.04km
Industry 11	Polymers	2.02km
Industry 12	Polymers	2.15km
Industry 13	Packaging	2.05km
Industry 14	Packaging	2.16km
Industry 15	Adhesives	2.38km
Industry 16	Polymers	2.60km
Industry 17	Polymers	2.63km
Industry 18	Polymers	3.13km
Industry 19	Chemistry industry	2.88km
Industry 20	Polymers	2.73km
Industry 21	Polymers	3.28km
Industry 22	Polymers	3.13km
Industry 23	Polymers	3.04km
Industry 24	Polymers	4.70km
Industry 25	Automotive products, oil	2.90km
Industry 26	Polymers	1.06km

Source: Authors.

Knowing the different scenario of each individual, to visualize how the localization and the season may change the deposition of TrOCs on *E. uniflora* leaves, some multivariate statistical analyses were performed (Figure 1).

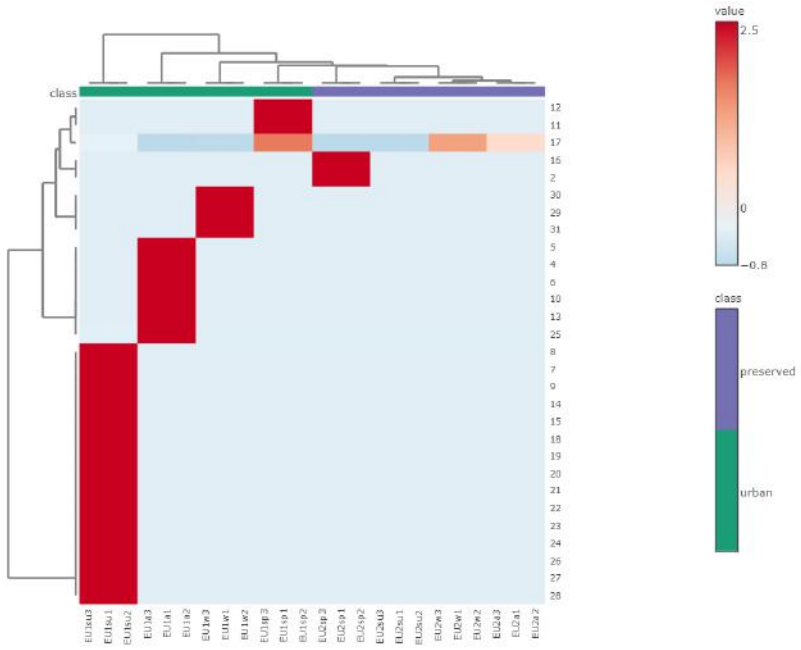
Figure 1 - Scatter plot generated by PLS-DA using the metabolomic data from the four seasons of the *E. uniflora* individuals.



Source: Authors.

The scatter plot generated by PLS-DA (Figure 1) shows that the TrOCs from individuals in the preserved and urban regions do not have overlapping characteristics and distribution, and therefore have definite chemical differences between them. The samples from the preserved and urban areas are in separate clusters, indicating a pattern between areas. The TrOCs from the preserved area fluctuate less due to abiotic factors. On the other hand, those from urban areas change more by season, because of the impact of human activity, like the flow of cars and the industrial operation in the area. The urban samples present more diversity and quantity of pollutants, as is seen on the heatmap (Figure 2)

Figure 2 - Heatmap of the TrOCs found in the leaves of *E. uniflora* from urban and preserved locations throughout the seasons.



Source: Authors.

Some of the organic contaminants were identified on the leaf surface of both individuals and can be related to the pollutant transport (Zalakeviciute *et al.*, 2018; Rahman *et al.*, 2022), such as butylated hydroxytoluene (17) which is widely used in packaging industries (Babich, 1982). There are four industries of this type near 2km of the individual EU1, and none near the EU2 individual, and this might be the cause of the highest concentration on EU1. Its byproduct 2,6-Di(tert-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one (16) was present as well only in the EU2.

However, some other contaminants were present only in the urbanized and industrialized areas. Compound 4 is an octane isomer obtained from petroleum distillation (Lukowitz *et al.*, 2006) and it could be related to polymers industries or road traffic. The phthalates, as the compounds 29 and 31 are widely used as plasticizers and, as they just appear in this individual, can be attributed to the twelve polymers industries close to 3.5km of the EU1. This may represent that the urban environment can contribute to pollutant emission and deposition on leaf surfaces.

Harmfulness of TrOCs identified in *E. uniflora* leaves for human health and the environment

Of the TrOCs identified in this study, most of them have been reported in the literature as harmful to human health and/or the environment.

The butylated hydroxytoluene, present in 9.44µg/mL in EU1sp, can induce tumors in mice with a concentration of 250mg/kg-day (Umemura *et al.*, 2001). Currently, butylhydroxytoluene is considered a major public health problem, with studies describing its presence in food, in human and animal adipose tissue, and in the aquatic environment (Castillo et al., 2011; Sanches-Silva et al., 2007; Conacher et al., 1986). Toxicological studies report the incidence of nephrotoxicity, pneumotoxicity, and hepatotoxicity in rats exposed to BHT (Al-Akid et al., 2001; Fries et al., 2004). The limit of use of this compound in

packaging is 5ppm and its intake can come to 2.0 to 6.0 µg/kg, but associated with butylhydroxyanisole, it's intake can come higher and become a concern motif (Babich, 1982; Suh, 2005).

The pollutant 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde has also been considered extremely dangerous for the aquatic environment and human health, demonstrating a genotoxic potential greater than butylhydroxytoluene (Nagai et al., 1993; Hernández et al., 2009).

Several studies describe the harmful effects of human exposure to VOCs, in particular, mutagenic, neurotoxic, genotoxic, and carcinogenic effects. Adverse effects on the environment have been also reported (Li et al., 2021; USEPA, 2022).

According to Globally Harmonized System data, 2-octanol is classified as hazardous to the environment and human health and safety data. To 1-dodecanol, limits and toxicity standards for this pollutant are not yet fully evaluated or available (USEPA, 2023a; USEPA, 2023b).

The presence of Linear alkyl benzenes (LAB) has been frequently detected in aquatic and terrestrial environments (Takada et al., 1987; Holt et al., 1992; Gledhill et al., 1991). Studies show that these compounds and their derivatives (linear alkylbenzene sulfonate) are toxic to the environment and human health, and this toxicity is strongly associated with the number, position, and length of alkanes attached to the phenyl moiety (Fernández et al., 2002; Pillard et al, 2001; Almeida et al., 1994; Mungray et al., 2009).

Quantitative risk and hazard assessment values and other toxicological data of undecane, 2,4-dimethyl are not available (USEPA, 2023c), but aldehydes/ketones from the class of TrOCs are associated with negative effects on human health and negative impact on the environment (Roba et al., 2014; Li et al., 2022).

Hexadecane poses a health risk and can be fatal if ingested and enters the airways (USEPA, 2023d).

Phthalate derivatives, such as Bis (2-ethylhexyl) phthalate, have been classified as bioaccumulative pollutants in the atmosphere, water, and soil. These compounds are considered teratogenic, mutagenic, and carcinogenic at low concentrations and have been reported as active endocrine (Net et al., 2015; Park et al., 2012; Szewczyńska et al., 2020; Kim et al., 2019).

Bis(ethyl-hexyl) phtalate, present in 9.33µg/mL in EU1w, in previous studies, showed cancer in rodents, and it's use in gloves and food was banished in Japan (Petersen, Jensen, 2010). This compound is present in food in the 825 mg/kg concentration and this value implied the intake estimation of 8µg/kg body-weight/day, with the tolerance consumption of 0.05µg/kg body-weight/day (Petersen, Jensen, 2010).

Tributyl acetylcitrate is classified as an environmentally persistent pollutant and a recent study confirmed that it has endocrine-disrupting activity and lethal toxicity in zebrafish and Japanese medaka (Horie et al., 2022). It's present in 11.01µg/mL in EU1w and have the acute toxicity in *Xenopus laevia* of 12mg/mL (Xu, Gye, 2018).

According to the results found in this study, it is concluded that the leaves of *E. uniflora* present TrOCs, even when cultivated in a preserved forest area, characterized by a lower incidence of atmospheric emissions by vehicles and industries.

Some of the TrOCs found were persistent to the climatic variations that occur during the different seasons of the year, such as, for example, the butylhydroxytoluene. Others are more susceptible to being deposited under the leaves in certain seasons of the year. Among these are 2,6-di-*tert*-butyl-4-(hydroxymethylene)-2,5-cyclohexandien-1-one predominant in the winter period; 1-dodecanol in summer; 2,4-dimethyl-undecane in the period corresponding to autumn and tributyl acetylcitrate in winter. All identified TrOCs and/or their class are reported in the literature as potentially toxic to human health and the environment.

After doing the content estimation and extrapolating to a more relatable scenario, like drinking tea or phytoterapics production, the content in the samples can come to the order of mg/mL, close or higher to the tolerance intakes and become a health concern. or even drinking TrOCs that are already prohibited in food.

4. Conclusion

The organic pollutants TrOCs are higly present in the surface of *Eugenia uniflora* leaves, as probably every leaf in urbanized areas. The presence of these compounds can vary, thanks to abiotic factors, with space, urban and preserved, and seasonally, by the seasons in the years. Furthermore, the urban individuals are those with the highest diversity and content of TrOCs, but fluctuate more due to abiotic factors as seen in PLS-DA. Comparing the toxicity data to the TrOCs content estimation, it becomes clear that some attention may be given to what plant material the population is consuming, and an effort should be made to minimize the presence of the TrOCs in medicinal plants, such as *E. uniflora*.

Acknowledgments

This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP Proc. Nº2016/05369-0).

Declarations

Ethical approval: This is an observational study. The Research Ethics Comitte of the School of Arts, Sciences and Humanity of the University of São Paulo has confirmed that no ethical approval is required.

Conflict of interests: All authors declare no conflict of interest in this paper.

Consent to participate: As no human individuals were objects of this study, no consent to participate is required

Consent to publish: As no human individuals were objects of this study, no consent to publish is required.

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