Concentrations and Transportation of Metal and Organochlorine Pollutants in Vegetables and Risk Assessment of Human Exposure in Rural, Urban and Industrial Environments (Bouches-du-rhône, France)

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Abstract

The bioaccumulation of metals (As, Cd, Co, Cr, Cu, Ni, Pb, Sb, V, Zn, Al, Fe) and organochlorine compounds (PCDD-Fs, and PCBs) was assessed in soils and vegetables of 3 sites of contrasted anthropogenic influence (rural and industrial-urban areas). Cultivated soils in industrial areas exhibited diffuse pollution in organochlorine pollutants (PCBs and PCDD-Fs). The pollutant levels encountered in vegetables were always lower than the regulatory or recommended values by EU. However, the contents measured in vegetables cultivated near industrialised areas were significantly higher than those observed in rural areas, this was notably the case for Co, Cd, Cr, Ni, Pb, V, NDL- and DL-PCB, PCDD and PCDF. The leaf pathway appeared as the main absorption pathway for many contaminants. The results suggested that population exposure to pollutants was mainly caused by the vegetable ingestion. In the vegetables and soils, the toxicity was mainly caused by the V, Co, Cd and Pb contents to which can be added As and PCDD-Fs for soils. Therefore, the proximity of vegetable crops to highly anthropised areas has led to long-term exposure of vegetables and soils to air pollutants, leading to an accumulation in the food-chain and thus a risk for human health.

Introduction

For several years, in many cities of France and Europe, urban agriculture has developed with the expansion of kitchen gardens associations. These associations provide ecosystem services to the city, such as food supply services, regulator services (rainfall, pests, temperatures), support services with the maintenance of favorable biological conditions (soil quality, carbon storage, pollination) or cultural services (recreation, pedagogy, landscape). Beyond the ecosystem services provided, this urban agriculture questions about the risks that their geographical situation may induce, in particular through pollution of soils (Schwartz, 2009 ; Schreck et al., 2011), atmosphere (Uzu et al., 2014 ; Xiong et al., 2014), or irrigation water (Raja et al., 2015 ; Khatri and Tyagi, 2015). The benefits and potential dangers remain barely informed, encouraging territorial authorities and scientific research to investigate this subject, such as through the recent French research programs (Chenot et al., 2010; Schwartz et al., 2012). Indeed, the urban garden soils act as a sink for pollutants from the atmosphere, irrigation water and agricultural inputs, as a consequence, they will facilitate their transfer to the subsoil, the groundwater and the vegetable biomass produced, and thus possibly induce harmful impacts on the human health (Hu et al., 2012; Goix et al., 2014 ; Xiong et al., 2016).

Besides, these urban or peri-urban gardens may be located near industrial complexes, which can represent a major additional source of pollution impacting the quality of soils and vegetables (Douay et al., 2008 ; 2009, El Hamiani et al., 2010). It is the case around the Gulf of Fos, highly industrialised and urbanised area of the south of France (Austruy et al., 2016, 2019; Ratier et al., 2018). This territory, located about 50 km west from Marseille, hosts the first commercial and industrial harbour in France and Southern Europe, with the western basins of the port of Marseille (GPMM) established in 1966 which includes many industrial sites, including 12 SEVESO « high threshold » sites (GPMM, 2018). The chemical, petrochemical, steel, metallurgical, gas or waste treatment industrie operations produce a variety of pollutants such as dioxins and furans (PCDD-Fs), non-dioxin-like and dioxin-like polychlorobiphenyls (NDL and DL-PCBs) or trace metals and metalloids (TMs). Yet, many associative garden structures have been created in recent years in this region, some associative structures being among the oldest at the national level, set up from 1976. At the same time, this region is first market gardening region in France and many croplands are located near industrial areas.

While many studies have addressed the problem of the metal pollutant accumulation in the food chain (Douay et al., 2009; De Temmerman et al., 2015), the information presently available about organochlorine pollutants are scants. Polychlorobiphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are families of persistent organic pollutants (POPs) comprising respectively 209, 75, and 135 congeners. Industrial processes (steel and coke industry, chemical factory) and incinerators are the main anthropogenic sources of PCDDs and PCDFs emitted into the environment as by-products, while PCBs were intentionally produced by industry as technical mixtures, for use as dielectric fluids, organic diluents, plasticizers, adhesives and flame retardants (INERIS, 2011). Although the use and production of PCBs has been banned for a few decades in USA and Europe, they are still widespread pollutants in air, soils, sediments and biota, especially in industrialised regions with incineration activities. They are lipophilic and bioaccumulate in the food chain, so the diet is the main route of exposure for humans (INERIS, 2011). The main issues with these pollutants are their extreme persistence in the environment, and their high toxicity to living organisms (Kana and Samara, 2018).

The work presented here thus aims to characterize the quality of the soils and vegetables produced in 3 sites of contrasted anthropogenic influence (rural and industrial-urban areas). The main objectives of this study include the atmospheric exposure assessment of metal and organochlorine pollutants of vegetable crops in heavily anthropised areas and the calculation of the risks for environmental and human health related to the ingestion of contaminated matrices (soil dusts and vegetables) by determining the estimated daily intake (EDI), hazard index (HI) and maximum allowable daily quantities of vegetables consumed (MDI) (Austruy and Dumat, 2014). It is based on the accumulation of organochlorine contaminants (NDL and DL-PCBs, PCDD-Fs) and metal(loid)s (Al, As, Cd, Co, Cr, Cu, Fe, Ni, Pb, Sb, V and Zn) in soils and vegetables. The purpose was to provide new data on metal, NDL and DL-PCB and PCDD-F levels in cultivated lands and vegetable crops in an industrial area, to study the possible role of specific sources of contamination, and to assess whether vegetable consumption constituted a health risk for residents in these areas. This study provides novel findings on occurrence and concentrations of these pollutants in the high anthropised environments.

Materials And Methods

2.1. Site location and culture conditions

This study was conducted in collective and private gardens in the South of France (Figure 1). The sites were chosen according to their environment and distance to major industrial installations. Urban-industrial sites were characterized by an urban environment near (< 10 km) industrial installations, and located in Fos-sur-Mer (A-site, plots A1 to A4) and Port-Saint-Louis-du-Rhône (B-site, plots B1 to B4). The rural sites represented by rural areas away from any urban center, major road, or industry (> 25 km) were located in Grans (C-site, plots C1 to C4). The study plots were located at average distances of 5.3, 8.3 and 25.7 km to the industrial harbour respectively for A, B and C sites.

In each site, four plots were selected and 6 lettuce plants (*Lactuca sativa var Capitata*) per plot were cultivated. The plants were divided into two rows, 30 cm apart from each other. The cultures were spread over 8 weeks from September to October 2014. Watering was done directly on the soil with the public water. No inputs were allowed during the cultivation. The weather conditions are presented in Figure S1 with the ombrothermic diagram as well as the wind rose covering the culture period. This period was affected by a strong N-NW wind, dominant in the

region, and east winds at a lower frequency. The daily temperatures varied between 7.4 and 31.2 °C with an average of 20.3 °C.

The physico-chemical parameters of soils (pH, organic matter, total nitrogen, C/N, CEC and texture) are presented in Table S1. Overall, the crop soils, enriched in organic matter, notably on the C-site, had an alkaline pH and a silty-sandy texture. The soil of the A-site was slightly more sandy while the soil of C-site was more acidic (7.6).

2.2. Harvesting, sampling and pre-treatment of soils and vegetables

At harvest, lettuces were sampled by separating plant tissues (roots and leaves) with a ceramic knife to prevent contamination. A soil sample was collected for each plant harvested.

In the laboratory, root and foliar tissues of each lettuce were cleaned coarsely then weighed to determine their total fresh biomass. Once the plant tissues were separated and cut into small pieces, composite plant samples were realised for each plot by selecting, after homogenisation and by the quartering method, 20 g FW (fresh weight) per plant for the leaves and 10 g FW for the roots. In total, for each study site, 4 composite samples of root and foliar tissues, corresponding to the 4 crop plots per site, were performed and weighed to determine their fresh mass. Subsequently, each composite sample was rinsed with ultrapure water. In order to remove the soil particles adsorbed to roots, the root samples were introduced into an ultrasonic bath (Fisherbrand - FB 15051) before rinsing. These composite samples were frozen at -30 °C and freeze-dried (-55 °C / 0.035 mbar, Christ alpha 1-4LD). The dry mass was then determined for calculating the vegetables water content. The samples were finally crushed to a fine powder using a crusher equipped with bowls and balls of zirconium to avoid any metal or organic contaminations (Retsch MM400 - frequency 25 Hz – 2.5 min). All samples were stored at -30 °C until analyses.

For the soil samples, a composite sample was performed for each plot by selecting by the quartering method, after homogenisation and removal of coarse constituents, 50 g of each sample (N = 4). A part of each composite sample was pre-treated for the analysis of organochlorine contaminants (PCDD-Fs, DL and NDL-PCBs), and a second for the analysis of TMs, major elements (Fe, Al) and physico-chemical parameters. While the fraction reserved for the analysis of organic pollutants was frozen (-30 °C), freeze-dried and then sieved to 2 mm, the second part, after determination of the fresh weight, was dried at 40 °C, weighed for the determination of the dry mass and sieved to 2 mm. About 5 g of the 2 mm fraction were selected, according to the quartering method, and crushed to fine particles (< 100 µm) using an agate mortar for analysis of TMs.

In total, for each site, 4 replicates of roots, leaves and soils were performed.

2.3. Sample preparation and analyses

2.3.1. Mineralisation and analyses of TMs in soil and vegetable samples

For the pseudo-total TM concentrations in soils, the soil sample were mineralised with aqua regia (1/3 HNO_3 and 2/3 HCl, AFNOR standard NF ISO 11466) in a microwave oven (March 5 CEM) according to the procedure described in Austruy et al. (2019). A certified soil sample, ERMCC141 (loamy soil), and a blank test were carried out for each mineralisation run.

The total mineralisation of vegetable samples, described in Austruy et al. (2019), was performed with nitric acid and hydrogen peroxide. A certified plant sample, ERMCD281 (Ryegrass), and a blank test were run together with each series of mineralisation.

The analysis of plant and soil samples was carried out by inductively coupled plasma - mass spectrometry (ICP-MS). The results of the standard plant and soil samples showed concentrations whose variations were less than 20 % from the theoretical concentrations. Detection limits range from 0.05 mg.kg^{-1} (As, Cd, Sb) to 0.1 mg.kg^{-1} (Al, Fe, Cr, Co, Cu, Ni, Pb, V and Zn).

2.3.2. Extraction and analyses of organochlorine contaminants in soil and vegetable samples

17 PCDD-F congeners listed by the World Health Organisation (Van den Berg et al., 2006) were analysed in this study. Their determination in soil and vegetable samples was performed by La Drôme Laboratoire (Valence, France) granted with the ISO 17025:2005. The ASE extraction (ASE300, Dionex) was carried out on 1 g of each sample with dichloromethane/acetone (50/50, v/v) and by running 3 cycles of 5 min at 120 bar and 100 °C. The samples were concentrated to 10 mL and treated with copper (12-h stirring). The extracts were spiked with corresponding 13C-labeled compounds and acidified with 2 mL of concentrated sulfuric acid (98 %). Then, the solution was purified using a Florisil 3 % cartridge, and the PCDD-Fs were eluted using toluene. A second purification was performed on a carbon-celite 18 % solid-phase cartridge. PCDD-Fs were separated by GC (Agilent 7890A) equipped with an apolar column (RTXPCB 40 m × 0.18 mm × 0.18 μm). The temperature program was as follows, 140 °C (hold for 0.6 min) increased to 210 °C at a rate of 35 °C.min^{-1} ; to 250 °C at 1.6 °C.min^{-1}; and finally to 310 °C at 3.5 °C.min^{-1}. The samples were detected by HRMS after electronic impact ionization (Jeol 800D). Blank tests and internal standards were included in the analyses to ensure the result quality.

For PCBs, a total of 18 congeners, defined as a priority by the European Commission, was analysed, 12 dioxin-like PCBs (DL-PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189) and 6 non-dioxin-like PCBs (NDL-PCB 28, 52, 101, 138, 153 and 180). These analyses were carried out by La Drôme Laboratoire (Valence, France). The ASE extraction (ASE300, Dionex) was carried out with dichloromethane/acetone (50/50, v/v) on 1 g of each sample. After purification with concentrated sulfuric acid and desulfurization, the PCBs contained in the extract were quantitatively determined by capillary-column gas chromatography with electron capture detector (GC/ECD). A blank test and internal standard were also analysed to check the conformity of the results.

For soils, the detection limits were 0.5 μg.kg^{-1} for PCBs and 0.5 ng.kg^{-1} for PCDD-F, and for vegetables, they were 2.5 ng.kg^{-1} for PCB and 0.25 ng.kg^{-1} for PCDD-F. Concentrations below the LOD were assigned a value of zero for statistical analysis.

2.4. Index calculation

To assess the TM distribution in the vegetables, the translocation factor was calculated (TF - Eq.1). The TF represents the transfer of the pollutant from the roots to the aerial parts of the vegetables. It is calculated from the pollutant concentrations measured in the different organs of plants (roots and leaves), as:

TF = C_l (mg.kgDW^{-1}) / C_r (mg.kgDW^{-1}) Eq.1

C_l corresponds to the leaf concentrations and C_r corresponds to the root concentrations.

The potential health risk associated to the ingestion of contaminated soils or vegetables for an adult can be assessed by calculating the estimated daily intake (EDI) of TMs, PCDD-F and PCBs using Eq.2 (Pouschat and Zagury, 2006; Austruy and Dumat, 2014):

$$EDI = C * IR / BW \quad Eq.2$$

Where EDI = potentially toxic element daily intake ($\mu g.kgBW^{-1}.d^{-1}$); C = exposure concentration of TMs, PCBs or PCDD-Fs ($\mu g.kgFW^{-1}$); IR = soil or vegetable ingestion rate ($kgFW.d^{-1}$); and BW = body weight (60.4 kg for a 18–79 year old adult, INSEE, 2006). For the calculation of EDIs, the adult IR was estimated to 130.9 g of fresh vegetables excluding potatoes which corresponds to 4.5 % of the total consumption over one day for an adult, which we extrapolated to lettuce as a worst case scenario (ANSES, 2017) and 0.1 g of soil dust which corresponds to 0.003 % of the total consumption over one day for an adult (US-EPA, 2008; ANSES, 2017). Noncarcinogenic hazards through vegetable ingestion were characterized using the hazard quotient (HQ, Xiong et al., 2016). It is defined as the quotient of the chronic daily intake, or the dose divided by the tolerable daily intake (TDI, $\mu g.kgBW^{-1}.d^{-1}$) of a specific compound (Eq.3, Luo et al., 2012; Uzu et al., 2014) calculated for each pollutant:

$$HQ = (EDI * EF * ED) / (TDI * AT) \quad Eq.3$$

EF is the exposure frequency (350 day/year for vegetable consumption in residential area, USEPA, 2008), ED is the exposure duration (83 years) equivalent to the average lifetime (INSEE, 2019), and AT is the averaging time for noncarcinogens risk (ED * 365 $day.year^{-1}$). TDI is a toxicological reference value "at dose threshold" defined, in the present case, for oral and chronic exposure (Table 3, INERIS, 2009). These values represent the quantity of a compound that can be ingested by humans without risk to health (expressed in $\mu g.kgBW^{-1}.d^{-1}$). To assess the overall potential for noncarcinogenic effects posed by more than one chemical, a Hazard Index (HI) was applied (Zheng et al., 2010). Although interactions between some pollutants could result in synergistic or antagonist effects (Fulladosa et al., 2005 ; Dondero et al., 2011 ; Yekeen et al., 2016), here, all the risks were assumed to be added (Cao et al., 2010; Luo et al., 2012 ; Liu et al., 2014). The HI was calculated from Eq.4:

$$HI = \Sigma HQ \quad Eq.4$$

An exposed population is assumed to be safe if HI < 1, otherwise, adverse health effects may be expected (Liu et al., 2014). The maximum allowable daily quantities of lettuce consumed were also calculated to provide suggestions to the local inhabitants. The maximum allowable daily plant intake (MDI, $gFW.d^{-1}$) to reach the TDI was therefore calculated as below (Eq.5):

$$MDI = (TDI * BW) / C \quad Eq.5$$

2.5. Statistical analyses

The interpretation of all data, especially the statistical analyses, was carried out using the R software (R Core Team 2015, version 3.2). Given the size of the samples per plot (4 replicates), the nonparametric Mann Whitney test, allowing the comparison of independent samples, was used to compare the distribution of data by station.

A principal component analysis (PCA, with standardized data) was performed with the quantitative descriptive parameters and the TM and organochlorine pollutant contents for soil and vegetable samples, for all the studied sites. In addition, to determine possible correlations between the different variables, the Spearman regression coefficients, a measure of non-parametric statistical dependence between two variables, were calculated.

Results And Discussion

3.1. TM concentrations in soils and vegetables

3.1.1. Concentrations in soils

The pseudo-total concentrations of TMs in the garden soils are presented in Table 1. Some TMs had concentrations higher than the pedogeochemical background measured on the territory (Austruy et al., 2016). This was the case for Cr, Cu, Pb, V and Zn in the three sites, for Cd in the A and B sites and Ni only in B-site. Thus, enrichment factors greater than 2 and synonyms of significant anthropogenic surface inputs (Redon et al., 2013) were measured for Cd, Zn and to a lesser extent Pb in the A and B sites, and Cu in the C-site. Finally, anthropogenic contributions of Zn were measured in one of the soils of C-site, with a high content at the surface (536 mgZn.kg^{-1}).

These inputs could have several origins. For Zn, Cd and to a lesser extent Pb, the anthropogenic contributions mainly concerned the south of the territory (A and B sites) and could therefore be the consequence of emissions from the industrial activities. Zinc, measured in high concentrations in one of the agricultural soils in C-site, was more likely originating from organic or mineral inputs previously used and enriched in Zn (Redon et al., 2013). Concerning Cu, the anthropogenic surface input on one plot on C-site could be explained by the fungicidal treatments previously provided to the cultures (Bordeaux mixture). The pseudototal TM concentrations of soils highlighted that the A and B-sites, located in the vicinity of the industrial installations, did not induce a global over-exposure of roots, compared to the more remote C-site. Overall, the soil concentrations remained at acceptable levels.

3.1.2. Bioaccumulation and translocation in lettuces

Table 2 presents the TM concentrations measured in the root and leaf tissues of the cultivated lettuces. For Pb, Cd, Co, Cr, As and V, the concentrations recorded were significantly higher in A and B sites than in the C-site. Whatever the TM, the maximum concentrations in the lettuce leaves were all observed in the B-site, except Co, V and Zn, which were higher in the A-site.

The TM concentrations observed in the lettuce leaves sampled in the A and B sites were, in many cases, higher than the usual concentrations in lettuces cultivated in France and intended for consumption (Table 2, ANSES, 2011). This was the case for Co, Cr, Ni, Pb and V which presented concentrations up to 3 times higher than usual French concentrations. However, despite high Pb and Cd contents, these did not exceed the maximum levels authorised in the leaf vegetable foodstuffs set at 0.3 and 0.2 mg.kgFW^{-1}, respectively (CE n° 1881/2006). On the contrary, the concentrations of As, Cd, Cu and Zn measured at the different sites were within the national value ranges. As indicated previously for soils, in the agricultural context, the fungicide treatments, organic fertilizers and animal effluents are important sources of inputs of these TMs in soils and plants (Mico et al., 2006; Komarek et al., 2008; Redon et al., 2013). Thus, being comparable, the concentrations of Cd, Cu, Zn, and to a

lesser extend As measured in this urban and industrial area remained limited if we refer to the usual French levels.

In A and B sites, the TMs were more concentrated in the leaves than in the roots with a TF greater than 1 (Figure S2A), except for As and Sb in both sites, Cd in the A-site and Cr in the B-site. Conversely, in the C-site, the TFs were often less than 1 indicating a preferential metal storage in the roots, as it was notably the case for As, Cd, Cr, Sb and Zn. The differences in the TM translocation in vegetables observed between the sites located near to industrial port zone of Fos (A and B sites) and the C-site, especially for Cd, Cr, Pb and V, highlighted the leaf exposure to air pollutants emitted by the industrial activities and the associated road and maritime traffic and thus the predominance of the foliar pathway in TM bioaccumulation for the A and B sites. The highest TFs were always observed on B-site, located southwest of the industrial harbor and exposed, notably under the dominant Mistral wind, to industrial emissions.

3.2. PCDD-F and PCB concentrations in soils and vegetables

3.2.1. Concentrations and congener profiles in soils

The studied organochlorine pollutants, polychlorobiphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), are generally classified as unintentional toxic and carcinogenic by-products, released from anthropogenic activities and, for PCDD-F, natural processes such as forest fires (Wikoff and Urban, 2013). The toxicity of PCDD-Fs and dioxin-like compounds is evaluated from 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), identified as the most toxic congener (Kumar et al., 2013; Gillbreath and McKee, 2015), from which is defined the toxic equivalency (TEQ) concept which is the basis for their health risk assessment (Van den Berg et al., 2006, Table S2).

The PCDD-F contents measured in garden soils are presented in Table 1. All congeners were detected in at least one soil sample. The concentrations in the soils varied from 18.9 to 1886.4 $ng.kgDW^{-1}$, recorded respectively in C and A-sites. Significant differences were observed for the PCDD-F contents between the 3 sites with average PCDD-F concentrations of 1303.3, 130.3 and 29.7 $ng.kgDW^{-1}$ measured in the soils of the A, B and C-sites, respectively. These mean levels expressed in TEQ were 7.04, 1.78 and 0.31 $ngTEQ.kgDW^{-1}$ in the A, B and C-sites, respectively. The PCDD-F contents recorded on these 3 sites were representative of the concentrations measured in soils in rural areas for C-site, in industrial areas including incineration activities for A-site, and urban soils for B-site (Bodenan et al., 2011, Urban et al., 2014).

The congener profile (Figure S3, Table S3) showed that there was no significant difference in the distribution of congeners and the proportion of dioxins and furans between the A and B sites (Kruskal-Wallis, n = 4, p > 0.05). On the contrary, the proportion of the furan congeners was significantly greater on the C-site compared to the two other sites, but its distribution may be biased by the low concentrations measured in this site. Furthermore, a greater diversity of PCDD-F congeners was observed in A and B sites compared to C-site (in average 16 congeners in A and B sites against 8 congeners in C-site). As in many studies (Jou et al., 2007; Bodenan et al., 2011; Prinz, 2017), the major congeners encountered in soils were octachloro-dibenzo-p-dioxin (OCDD) and octachloro-dibenzo-p-furan (OCDF) followed by 1,2,3,4,6,7,8-HpCDD and 1,2,3,4,6,7,8-HpCDF (Figure S3). Hepta- and octa-PCDD-F congeners are generally associated with emissions from uncontrolled temperature sources, such as inefficient biomass combustion and/or open burning of wood and household waste (Kouimtzis et al.,

2002). Likewise, the steel industry and coke plants are known as major sources for PCDD-Fs, and are often responsible for the emission of a greater congener diversity, notably due to the use of electric arcs and basic oxygen furnaces (Buekens et al., 2001 ; Leung et al., 2007 ; Zubair et Adrees, 2019).

Due to their higher toxicity and despite their low concentrations, the congeners 1,2,3,4,6,7,8-HpCDD, 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF represented more than 40 % of the toxicity measured for all dioxin-like congeners, while OCDD, the most concentrated congener in the soils (representing between 62 and 78 % of total PCDD-Fs), contributed to less than 5 % of the toxicity. Similarly, while dioxins were predominant in soils, the toxicity was mainly caused by the furan congeners (Figure S3 and Table S2). The concentrations and distributions of PCDD-F congeners in the studied soils highlighted the impact of the industrial activities in particular steel and incineration activities. The reduction of PCDD-F concentrations in soils with distance from the industrial port zone reflected the dilution of diffuse pollution with distance to the emission sources.

The contents of NDL-PCBs and DL-PCBs measured in the different soils are presented in Table 1. PCBs were found only in soils located near to the industrial area, such as in the A-site with very variable levels (between 5 and 394 µg.kgDW^{-1}, for DL+NDL-PCBs), and at low concentrations in two of the 4 soils of B-site (2.8 µg.kgDW^{-1}, in average). The PCBs encountered were essentially NDL-PCBs (between 96 % and 100 %), only two DL-PCBs, PCBs 118 and 156, were detected in small quantities in the soils of A-site. The congener distribution measured in this site showed a predominance of hexa-CBs (57.9 %) and to a lesser extent hepta-CBs (33.6 %) (Table S3). The profiles of PCB congeners also highlighted a major contribution from PCB 153 (33.6 %), PCB 180 (32.1 %) and PCB 138 (25.1 %). These results might relate to the mode of deposition of PCBs on the soil. The least chlorinated congeners can be transported over longer distances to remote sites because they generally remain in the gas phase (Kumar et al., 2013) unlike the heaviest. This distribution could be also the consequence of a former soil contamination of PCB, the main route of PCB elimination from soils being volatilisation, which only affects the lightest congeners (Motelay-Massei et al., 2004; Colombo et al., 2013; Vane et al., 2014). The existence of nearby sources of industrial emissions could constitute the main cause of soil pollution around the Gulf of Fos (Wang et al., 2011a).

3.2.2. Organochlorine pollutant levels in lettuces

The concentrations of NDL-PCBs, DL-PCBs and PCDD-Fs in the leaves and roots of lettuce cultivated on the 3 sites are presented in Table 2 and compared to the usual values recorded in green vegetables in France by ANSES (2011). The highest concentrations for these compounds were measured near the industrial port area, in the A and B sites, but the concentration values never exceeded the action levels set out in the EU Recommendations (2014/663/EU). The lettuces grown in the A-site had the highest levels of PCDD-F, while the NDL- and DL-PCB contents were within the same value range for both sites.

There are still few studies on the accumulation of organochlorine pollutants in leafy vegetables, and the few existing studies differ in units of measurement and congeners. By way of comparison, two studies carried out in industrial regions of Italy on different vegetables including lettuce (Grassi et al., 2010; Esposito et al., 2017) showed concentrations of PCDD-F, DL and NDL-PCB in the same range as those recorded in the present study, in particular for A and B sites close to the industrial port zone of Fos. In addition, a previous study carried out in France near a waste incinerator (INSERM, 2000) revealed average concentrations of PCDD-F in lettuce of 1.1 ngTEQ.kgDW^{-1}, higher than levels measured on all study sites. On the contrary, studies carried out in China in

urban areas (Zhang et al., 2008) or throughout Greece (Papadopoulos et al., 2004) recorded lower PCDD-F contents, on average 0.036 ng.kgFW^{-1} in the first case and 3.55 ng.kgDW^{-1} in the second case, than those recorded in our study area and in particular those recorded on A-site (1.38 ng.kgFW^{-1} or 25.79 ng.kgDW^{-1} of PCDD-F).

In detail (Table S3), the predominant PCB congeners measured in vegetables were PCB 101, 138, 153 and 180, generally considered as characteristic of industrial emissions (Grassi et al., 2010; Wang et al., 2011a), except in the C-site where a lower proportion of PCB 180 was observed against a higher proportion of weakly chlorinated PCBs (PCB 28 and 52). The highly chlorinated PCBs were predominant in A and B sites close to the industrial harbour. Regarding PCDD-F congener profiles (Figure S3), the dominant congeners in vegetables were, as for soils, OCDD, 1,2,3,4,6,7,8-HpCDD and 1,2,3,4,7,8,9-HpCDF, the toxicity being mainly brought by 1,2,3,4,6,7,8-HpCDD and 1,2,3,4,7,8,9-HpCDF. Unlike the soil, the toxicity, in toxic equivalent, in the lettuce leaves was mainly driven by DL-PCBs and more particularly by PCB-126 in the A and B sites.

Figure S2B presents TF calculated for PCDD, PCDF, NDL-PCB and DL-PCB. PCDD-Fs were mainly stored in the roots, while PCBs were distributed between leaves and roots in function of chlorinated degrees notably (Figure S4). Indeed, the leaf/root translocation factor for PCB increased with the degree of chlorination, whatever the study site, highlighting a preferential storage of highly chlorinated PCBs in the aerial parts. This is the sign of an atmospheric exposure, corroborating that the leaf pathway should be the main route of PCB absorption (INERIS, 2011). The weakly chlorinated PCBs, 3-PCB, 4-PCB and to a lesser extent 5-PCB, which can be absorbed by roots due to their low molecular weight, were preferentially stored in the roots in all sites. A high proportion of weakly chlorinated PCBs was observed in the lettuces of the C-site, 14.1 and 9.9 % for 3-PCB and 18.2 and 13.1 % for 4-PCB for root and leaf tissues respectively, compared to the part which represent on all congeners (6 and 17 % for 3-PCB and 4-PCB respectively). On the contrary, the proportion of high molecular weight PCBs, 6-PCB and 7-PCB, in the A and B sites, on average greater than 50 % and close to 15 % respectively, was much higher than the share they represent on all congeners (33 % and 11 % respectively for 6-PCB and 7-PCB). This reinforced the hypothesis that the leaf pathway was predominant in the absorption of PCBs, especially for the most chlorinated. Furthermore, these results confirmed the exposure of areas located near the industrial harbour of Fos (A and B sites) to current PCB emissions (waste incinerator, petrochemical and steel plant, ...) whose reactivity in air and the faster oxidation of low chlorinated PCBs could have led to a predominance of the most chlorinated (Gambaro et al., 2004; Wang et al., 2011a). The congener profile encountered in vegetables of C-site, with a lower proportion of highly chlorinated PCBs, might be the consequence of a greater distance from the sources of industrial emissions, allowing for a dilution of atmospheric concentrations and degradation of the most chlorinated PCBs.

3.3. Sanitary risks

The values of EDI, MDI and HI of pollutants in soils and vegetable leaves are listed in Table 3. The EDI calculated for metal and organochlorine pollutants on vegetable and soil matrices did not exceed the TDI in any site. In lettuce leaves, the highest EDIs were measured in the A-site except for Cd, Cu, and total PCBs for which the maximum values were recorded in B-site. For Cd, Cr, Co, Ni, Pb, V, total PCBs, PCDD-F and DL-PCB, the EDI were thus significantly higher in the A-site compared to the C-site. Similarly, for some compounds (Cd, Cr, Ni, PCBtot, PCDD-F), the EDI were significantly higher in the B-site compared to the C-site. The EDI were lower for soil ingestion than for vegetable consumption and no significant difference was observed between the sites for

the soil matrice. Consequently, the HIs did not exceed 1 whatever the matrix and the pollutant considered except for the lettuces in one garden plot of the A-site (HI=1.03), in relation with the high HQ of Co, Pb and V. The maximum allowable daily lettuce intake (MDI) that could be ingested without risk to health varied depending on the site and the contaminant, it was about 670 gFW.d^{-1} for lettuces cultivated in A-site, 706 gFW.d^{-1} for lettuces of B-site and nearly 1 400 gFW.d^{-1} for those cultivated in C-site. The limiting contaminants depended on the site but were always a TM, V for A-site, Cd for B-site and Co for C-site.

Among the studied pollutants, HQs of V, Co, Cd and Pb were the highest in the vegetables of A and B sites. Thus, the HI was mainly composed of the HQ of V (11.8 - 19.2 %), Co (8.2 - 24.9 %), Cd (6.8 - 25.1 %) and Pb (7.1 - 12.2 %). It should be noted that Co, Pb and V concentrations in lettuce leaves of the A and B sites were above the usual concentrations measured at the national level, confirming an overexposure to these elements by a repeated ingestion. The QG of Cr were low whereas the concentrations measured in lettuce leaves in A and B sites were significantly higher than the French national levels (ANSES, 2011). This was mainly due to its high oral reference dose (Cr(III), 300 µg.kgBW^{-1}.d^{-1}). The TDI of Cr(III) was used to represent that of total Cr in this study because Cr(VI) is reduced to Cr(III) under the acidic conditions in the stomach (Wang et al., 2011b). Regarding organochlorine contaminants, the cultivated lettuces provided a limited contribution to the tolerable dietary PCB intake. The EDI of PCBs through this food item was maximum 0.7 % of the TDI in adults, while the EDI of DL-PCBs and PCDD-F was maximum 7.4 % of the TDI. Thus, these results indicated a higher potential health risk from the lettuce ingestion for Cd, Co, Pb and V and in general for TMs compared to organochlorine pollutants. This was confirmed by the EDI for these elements which represented on average, on the A-site, 7.4, 12.0, 6.5 and 13.4 % of the TDI respectively for Cd, Co, Pb, and V while the share of vegetables in human diet was evaluated at 4.5 %. Despite lower HIs, the same trend was observed for soils whose the toxicity mainly driven by V, Co, Cd and Pb contents to which can be added As and PCDD-F, the latter representing on average 31.6 and 9.4 % of HI, respectively. However, while the estimated average quantity of soil ingested per day represents 0.003 % of the total daily consumption for an adult, EDI of all pollutants, notably for the A and B sites, were greater than 0.003 % of the TDI and could reach 2.63 % for As in A-site. This means that soil ingestion implied an excessive intake of these pollutants and could provide up to 800 times more pollutants compared to which soil ingestion represents in the human diet.

A and B sites thus recorded the most important HI highlighting a higher risk for human health in the event of ingestion of soil dusts or vegetables produced in market garden areas exposed to industrial emissions. This was in agreement with other studies (Grassi et al., 2010; Xiong et al., 2016), which suggested that when market garden and crops were set up in the vicinity of industrial activities, long-term exposure could lead to an increased accumulation of pollutants. Consequently, it would affect the accumulation of pollutants through the food-chain, and finally increase the sanitary risks. While the interactions between pollutants, which may be different depending on the matrix considered, may lead to synergistic or antagonistic effects (Yekeen et al., 2016), the health risk was assessed from the cumulative effects via HI, a method which can therefore under or over assess the health risk. In light of HIs, the results suggested that population exposure to pollutants was mainly caused by the ingestion of vegetables. We thoroughly washed the lettuce to minimize leaf surface contamination by soil particles, which means that we probably underestimate the risk associated to vegetable ingestion. Indeed, Schreck et al. (2012) demonstrated that careful washing before lettuce ingestion removes up to 25 % of the total metal-rich particles.

3.4. Impact of industrial activities on pollutant bioaccumulation in vegetables

Figure 2 presents a principal component analysis (PCA) carried out with the concentrations of TMs, PCDD-F, DL-PCB and NDL-PCB measured in lettuces, leaves and roots, cultivated in the different sites. The first two axes of this PCA explained 61 % of the variance. The first axis, representing 45 % of the data variability, allowed for distinguishing PCBs and the majority of TMs, except As, from PCDD-F with a strong influence of Pb, Co, Cd, Ni, Cr and V. Axis 2, which represented 16 % of the data variability, has differentiated 3 groups, PCBs, PCDD-F and As, and remaining TMs.

The distribution of concentrations measured in the leaves and roots were influenced by the two axes, each reflecting a separate aspect of the bioaccumulation pattern. First, axis 1 allowed a spatial differentiation, the vegetables of B-site and to a lesser extent of A-site being mainly correlated with the TMs and PCBs, unlike the vegetables of C-site. This axis illustrated the variation among sites in terms of exposure to pollutants. Secondly, the concentrations observed in the lettuces followed a distribution according to the plant tissue along the axis 2. It informed about the different pollutant storage location in the vegetables, which was partly dictated by the absorption and exposure pathways. These results suggested a preferential exposure of the aerial parts to some metals, such as Pb, Co and Cd, recognized as the main pollutants emitted by industrial activities in this study area (Sylvestre et al., 2017; Ratier et al., 2018; Austruy et al., 2019), and to PCBs, for which the foliar uptake was predominant especially for the most chlorinated (Grassi et al., 2010). Indeed, the root uptake of PCBs is limited due to their high adsorption capacity on organic matter and clays of soil, their shape, their weight and their hydrophobic character (Quéguiner et al., 2010; Mitra et al., 2019). On the contrary, root transfer seemed to be the main pathway for As, for which the soils of the three sites showed comparable levels and whose root absorption has been documented in many studies (Kumpiene et al., 2012; Austruy et al., 2013, Kumpiene et al., 2021), and for PCDD-F, whose root uptake is recognized as the main pathway in plant species (Zhang et al., 2009). The contents measured in the leaves may have been absorbed by the foliar pathway as a result of atmospheric exposure to these compounds. It has also been pointed out that the root uptake of PCDD-F was restricted to the root system and could not be translocated to the aerial parts (Engwall and Hjelm, 2000), which means the contents measured in the leaves may have been absorbed by the foliar pathway as a result of atmospheric exposure to these compounds.

These results were confirmed by the existence of significant correlations between the concentrations of many pollutants measured in the aerial parts of vegetables (Cd, Co, Cr, Ni, Pb, V, NDL-PCB, DL-PCB and PCDF) and the distance to the industrial port zone of Fos (Table S4). Unlike PCDDs, which have shown no significant correlation in soils or vegetables with the distance to industries (R = -0.55 and -0.40, p > 0.05), PCDF levels were strongly correlated (), allowing to consider the industries as the main emitters of these compounds in the sector. Previous studies (Gunes et al., 2014 ; Ratier et al., 2018) have shown that the industrial sector led to greater emissions of PCDF than of PCDD, as observed in this study. Thus, the TM, PCDF and PCB concentrations measured in the leaves of lettuces cultivated on the different sites corroborated the lower atmospheric exposure to these pollutants with increasing distance to the industrial harbour.

Conclusion

The vegetable gardens, increasingly developed in urban areas in France and in Europe (Galt et al., 2014), are often located in direct proximity to roads and / or industrial facilities (Pierart et al., 2015), and are, moreover,

threatened by the decrease in the surface area of agricultural land and their production capacity due to the expansion of cities and real estate pressure.

This study provided new data on the levels of TMs, DL and NDL-PCBs, PCDDs and PCDFs in vegetables and cultivated soils, useful for evaluating the food intake of these contaminants by the general population near industrial zones. The proximity of vegetable crops to highly anthropised areas such as the industrial-port zone of Fos has led to long-term exposure of vegetables and soils to air pollutants, leading to an accumulation in the food chain, finally reaching human exposure and therefore, a risk for human health. The consumption of vegetables cultivated near to an industrial area may thus represent a significant route of the intake of Cd, Co, Pb and PCDD-F, pollutants known for their toxicity at low doses of exposure. This work also provides details on the absorption pathways and storage of pollutants in vegetables, in particular organochlorines which are still poorly known, with a preferential storage of PCBs in the aerial parts whereas PCDD-Fs are mainly stored in the roots.

These results underline the importance of studying the behavior of pollutants in the environment and of assessing the risks they present to human health. Thus, the study of the accumulation and transfer of TMs and organic pollutants in vegetables in areas heavily impacted by human activities is a crucial issue for health.

Declarations

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Tables

Table 1: Concentrations of TMs, NDL-PCBs, DL-PCBs and PCDD-F in the various soils (n = 4).

	Units	A-Site	B-Site	C-Site
As	mg.kg^{-1} DW	**8.17** ± 0.78	**8.61** ± 0.76	**7.83** ± 2.90
Cd		**0.68** ± 0.07	**1.09** ± 0.32	**0.27** ± 0.05
Co		**6.99** ± 2.47	**7.51** ± 0.51	**6.14** ± 2.22
Cr		**29.75** ± 5.53	**30.59** ± 1.86	**30.35** ± 7.51
Cu		**18.86** ± 11.53	**17.50** ± 1.82	**23.06** ± 12.44
Ni		**20.33** ± 5.47	**21.66** ± 2.10	**20.77** ± 6.98
Pb		**23.09** ± 5.77	**28.69** ± 2.75	**21.71** ± 10.78
Sb		**39.42** ± 9.81	**41.18** ± 1.94	**36.75** ± 11.25
V		**32.90** ± 8.64	**38.06** ± 2.88	**35.00** ± 11.61
Zn		**72.86** ± 38.24	**96.50** ± 31.76	**180.35** ± 78.95
Al	g.kg^{-1} DW	**89.59** ± 23.62	**114.47** ± 9.33	**107.34** ± 34.42
Fe		**41.61** ± 12.00	**46.04** ± 2.04	**36.44** ± 14.06
PCDD	ng.kg^{-1} DW	**1038.75** ± 599.41	**107.28** ± 19.52	**24.36** ± 13.10
PCDF		**104.31** ± 53.07	**22.31** ± 4.32	**7.26** ± 2.12
NDL-PCB	µg.kg^{-1} DW	**142.00** ± 80.70	**2.75** ± 2.20	**0.00** ± 0.00
DL-PCB		**6.25** ± 5.10	**0.00** ± 0.00	**0.00** ± 0.00
PCDD-F+PCB-DL	ITEQng.kg^{-1} DW	**7.04** ± 2.09	**1.78** ± 0.98	**0.31** ± 0.45

Table 2: Minimum and maximum concentrations of metals, PCDD-Fs, NDL-PCBs and DL-PCBs (n = 4) at each site measured in the roots and leaves of the lettuces cultivated in the study sites compared to the usual French concentrations in lettuce (metals) or green vegetables (PCDD-F and DL and NDL-PCB) (n = 16 and 3 for the metal and organochlorinated compound concentrations, respectively - ANSES, 2011). In bold, the concentrations of the lettuce leaves equal or higher than the usual French levels.

	Units	France	A-Site		B-Site		C-Site	
		Leaves	Leaves	Roots	Leaves	Roots	Leaves	Roots
As	mg.kg^{-1} FW	0.000	0.003	0.014	**0.003**	0.061	0.000	0.005
		0.037	0.006	0.164	**0.038**	0.095	0.003	0.122
Cd		0.007	0.004	0.009	**0.009**	0.012	0.000	0.009
		0.037	0.015	0.057	**0.038**	0.042	0.002	0.020
Co		0.003	**0.023**	0.015	**0.019**	0.018	**0.007**	0.014
		0.023	**0.091**	0.120	**0.083**	0.027	**0.043**	0.049
Cr		0.027	**0.164**	0.133	**0.247**	0.152	0.017	0.112
		0.265	**0.604**	1.015	**0.814**	1.691	0.143	0.399
Cu		0.303	0.277	0.390	0.349	0.646	0.200	0.299
		1.630	0.446	0.931	0.928	1.612	0.487	1.902
Ni		0.000	**0.172**	0.111	**0.183**	0.118	0.034	0.049
		0.181	**0.242**	0.620	**0.431**	0.740	0.119	0.235
Pb		0.000	**0.041**	0.027	**0.053**	0.037	0.010	0.007
		0.067	**0.098**	0.114	**0.142**	0.131	0.059	0.073
Sb		0.000	0.000	0.003	0.000	0.003	0.000	0.000
		0.003	0.002	0.010	0.002	0.009	0.002	0.004
V		0.000	**0.062**	0.039	**0.062**	0.049	0.022	0.012
		0.087	**0.185**	0.409	**0.194**	0.165	0.091	0.130
Zn		0.792	1.364	2.048	**1.138**	1.426	0.226	2.359
		3.460	3.514	5.201	2.703	12.859	3.380	7.641
NDL-PCB	ng.kg^{-1} FW	22.11	**28.80**	25.35	**46.87**	78.35	11.96	10.14
		42.36	**50.57**	52.76	**53.99**	80.33	22.46	17.92
DL-PCB		4.21	**5.21**	6.54	**6.66**	13.96	2.95	4.18
		8.12	**12.29**	17.92	**9.26**	18.49	4.83	6.71
PCDD		0.04	**0.43**	0.66	0.08	0.37	**0.09**	0.69
		0.11	**3.13**	8.34	0.10	0.48	**0.21**	1.24
PCDF		0.04	**0.13**	0.19	0.07	0.30	0.00	0.00
		0.13	**0.33**	1.00	0.08	0.32	0.00	0.00
PCDD-F	pgTEQ.kg^{-1} FW	-	2.14	1.80	0.70	1.86	0.04	0.09

PCB-DL	21.41	30.21	0.80	2.01	0.55	5.46

Table 3: Tolerable daily intake (TDI – µg/kgBW/d) established by INERIS (2009) and the estimated daily intake (EDI – µg/kgBW/d), hazard index (HI) and maximum allowable daily quantities of vegetables consumed (MDI – g/d) calculated for trace metals, the sum of total PCB and sum of DL-PCB and PCDD-F (in TEQ), between brackets is indicated the limiting pollutant.

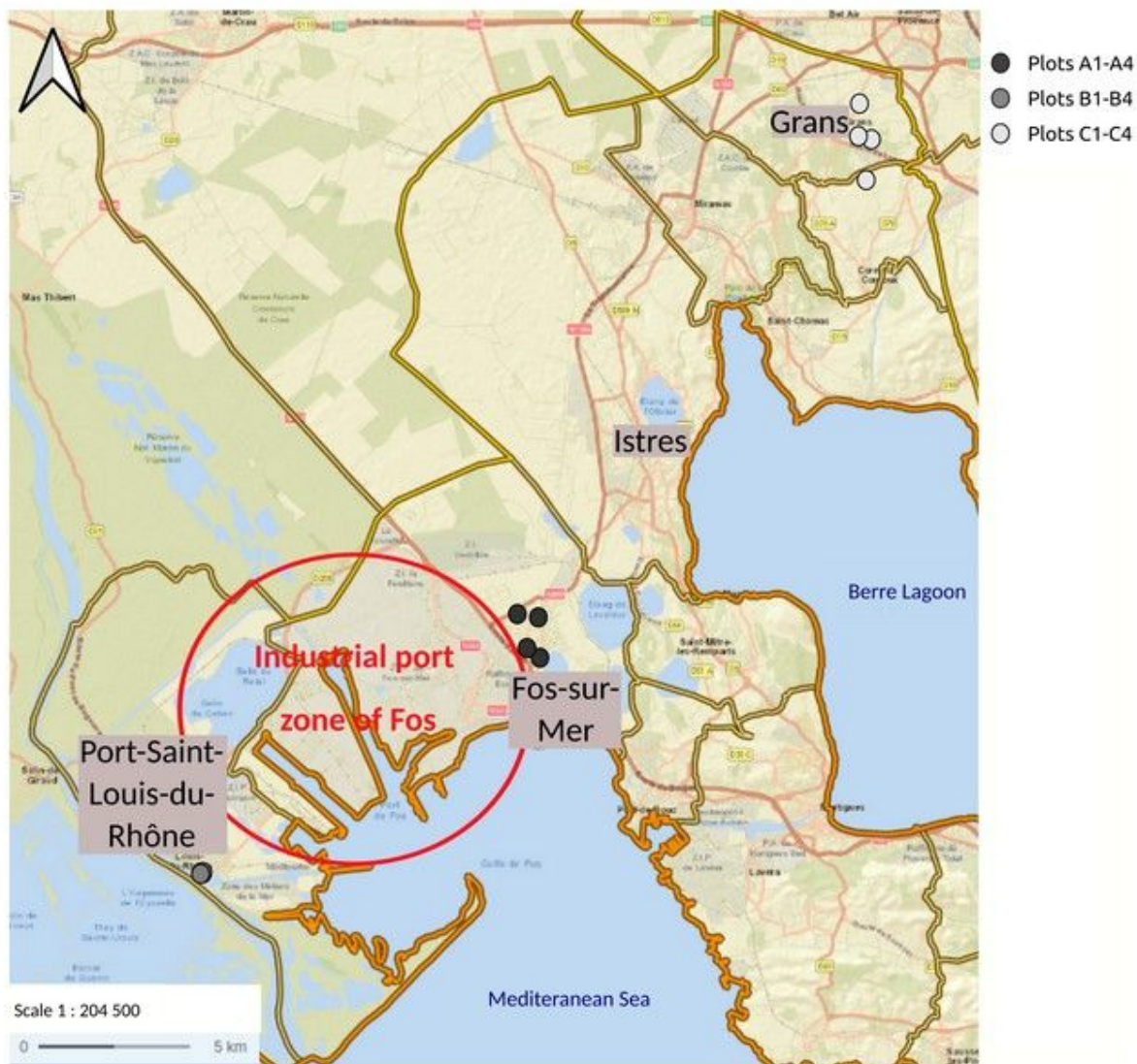|		A-site		B-site		C-site	
	TDI	EDI					
		Soils	Vegetables	Soils	Vegetables	Soils	Vegetables
As	0.45	0.01±0.00	0.01±0.01	0.01±0.00	0.01±0.00	0.01±0.00	0.00±0.00
Cd	0.36	0.00±0.00	0.03±0.01	0.00±0.00	0.04±0.03	0.00±0.00	0.00±0.00
Cr	300	0.04±0.01	1.00±0.65	0.04±0.00	0.66±0.29	0.04±0.00	0.19±0.17
Co	1.40	0.01±0.00	0.17±0.07	0.01±0.00	0.10±0.05	0.01±0.00	0.06±0.05
Cu	140	0.03±0.02	1.10±0.26	0.02±0.00	1.13±0.47	0.03±0.02	0.91±0.45
Ni	20.0	0.03±0.01	0.53±0.17	0.03±0.00	0.47±0.25	0.03±0.01	0.19±0.13
Pb	3.50	0.03±0.01	0.23±0.07	0.04±0.00	0.17±0.08	0.03±0.01	0.08±0.07
Sb	6.00	0.06±0.01	0.00±0.00	0.05±0.00	0.00±0.00	0.05±0.02	0.00±0.00
V	3.00	0.05±0.01	0.40±0.18	0.05±0.00	0.23±0.11	0.05±0.02	0.12±0.11
Zn	300	0.11±0.06	6.90±2.60	0.12±0.04	3.28±1.38	0.24±0.32	3.49±2.88
PCBtot	0.02	$2.8E^{-7}±2.2E^{-7}$	$1.2E^{-4}±0.3E^{-4}$	0.00±0.00	$1.3E^{-4}±0.1E^{-4}$	0.00±0.00	$4.2E^{-5}±1.2E^{-5}$
PCDD-F + DL-PCB	$7.0E^{-7}$	$8.0E^{-9}±0.00$	$3.1E^{-8}±1.6E^{-8}$	0.00±0.00	$1.5E^{-8}±0.2E^{-8}$	0.00±0.00	$0.1E^{-8}±0.0E^{-8}$
HI		0.29±0.06	0.71±0.27	0.29±0.02	0.48±0.23	0.25±0.09	0.23±0.17
MDI		3.6 (As)	670.4 (V)	3.9 (As)	705.5 (Cd)	3.2 (As)	1371.6 (Co)

Figures

Figure 1

Location of the different study plots in three sites of the Aix-Marseille-Provence Metropole (France). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
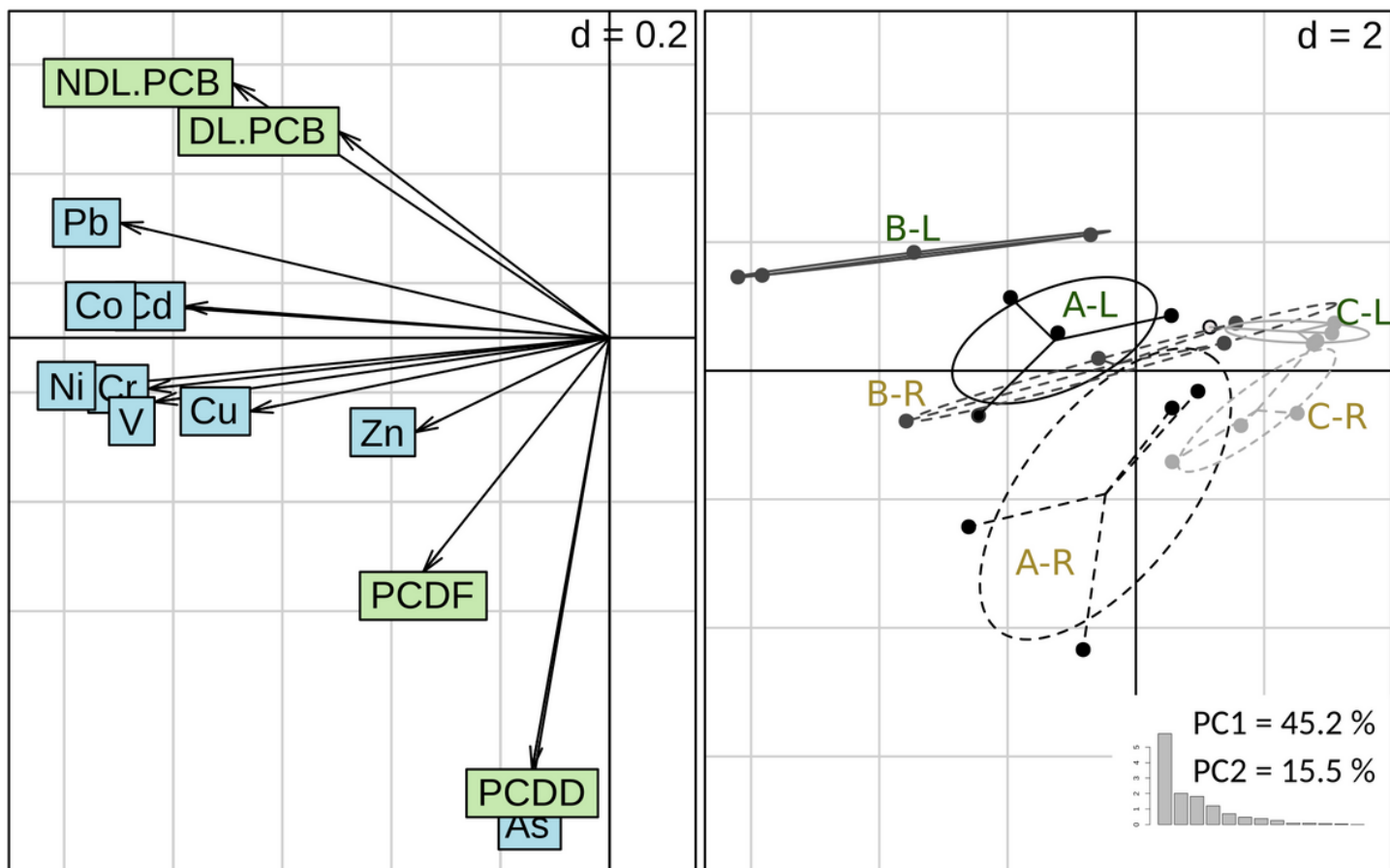
Figure 2

Principal component analysis (PCA) on the concentrations of metals, PCDD, PCDF, DL-PCB and NDL-PCB in leaves (L) and roots (R) of lettuces (n = 24). The solid lines represent the contents in the leaves, the dotted lines represent the contents in the roots.

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