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RESEARCH ARTICLE

Chemical characterization and phytotoxicity assessment of peri-urban soils using seed germination and root elongation tests

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Abstract

The peri-urban soil is exposed to pollutants because of its proximity to the city, which may influence the quality of agricultural products. In this study, the occurrence of 16 trace elements (TEs), 16 polycyclic aromatic hydrocarbons (PAHs), and 33 contaminants of emerging concern (CECs) was analyzed in two soil sites of the peri-urban area of Barcelona (Spain) (S2 and S3) and a pristine site (S1). Levels of Pb (S2 164 and S3 150 mg kg^{-1}) are around 2.5 times higher than the guideline values. Values for Cu (178 mg kg^{-1}) in S2 are 1.8-fold higher, whereas for Zn, levels are slightly above the threshold in S2 (208 mg kg^{-1}) and S3 (217 mg kg^{-1}). The total concentrations of PAHs are significantly below the limits: 24 ng g^{-1} dw (S1), 38 ng g^{-1} dw (S2), 49 ng g^{-1} dw (S3), whereas only some CECs are detected with low concentrations. We also developed a simple and rapid method to assess soil pollution. Here, we use two plant growth indexes (seed germination rate and root elongation at the initial stage) of three seeds (lettuce, tomato, and cauliflower) to assess soil chemical contamination on agriculture. In the peri-urban soil, the concentration of Pb was 2.5 times higher than the guideline values, whereas for Cu and Zn, values were slightly above their limits, while only few PAHs and CECs were detected. Results for principal component analysis suggest that root elongation is a more sensitive measurement endpoint than germination rate, especially for lettuce. The germination rate of tomato relied on the nitrate in the soil and decreased sharply in the site with pollution of Cu and As. Under the specific conditions of this study, cauliflower should not be recommended to assess environmental pollution due to its low sensitivity to pollutants. In conclusion, this is a low-cost, simple, and rapid method for evaluating the effects of chemical pollution of agriculture soils on seed growth.

Keywords Peri-urban soil · Trace elements · Polycyclic aromatic hydrocarbons · Contaminants of emerging concern · Seed germination rate · Root elongation

Introduction

The expansion of large cities has increased the demand for food (Ferreira et al. 2018; Tan et al. 2005). Among lots of solutions, peri-urban agriculture seems to be a favorable choice, since it minimizes the carbon dioxide footprint in terms of food transportation, helps recycle urban reclaimed water and biosolids, and furthermore offers fresher food products to the adjacent city (Duvernoy et al. 2018; Zasada 2011). However, compared with traditional rural agriculture, the peri-urban ecosystem inevitably faces numerous additional pressures and tensions for two interacting reasons. Firstly, a number of contaminations from large infrastructures (e.g., solid waste incineration, airports, highways, industrial emissions) are discharged into the peri-urban area. Secondly, large-scale vegetable production brings intensive use of fertilizers, pesticides, and reclaimed water, which also contributes to an important source of pollution (Margenat et al. 2017, 2018).

Soil is an important medium for plant growth; however, once it is polluted, some contaminants can be uptaken by crops and furthermore threaten human health. Therefore, when promoting peri-urban agriculture, it is quite necessary to consider the extent of soil contamination.

Among numerous classes of pollutants posed to the peri-urban soil (inorganic and organic), trace elements (TEs) have been monitored over decades years, since they are closely related to urban development. For example, Kabata-Pendias

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(2011) observed that from 1977 to 1997, in Warsaw (Poland), the concentrations of Li, Ni, Zn, Cr, Pb, Ba, Sr, and Fe in soil have increased sharply due to the extensive use of vehicles. Huang et al. (2018) reported that compared with background levels, the concentrations of many TEs in peri-urban soil increased significantly, especially Pb, with levels even higher than the Chinese legislative limits. In addition, Hu et al. (2018) revealed that surrounding land use and agricultural activities have a significant influence on TEs accumulation in peri-urban soil.

Polycyclic aromatic hydrocarbons (PAHs) are a large group of mutagenic and carcinogenic compounds with two or more fused aromatic rings. It was proved that marked increases of PAHs in soil following industrial revolution are mainly influenced by human activities, especially the incomplete combustion of organic materials in the urban area (Kim et al. 2018; Wilcke 2007), such as industrial production, residential heating, power generation, and vehicular emissions (Hussain and Hoque 2015; Moore et al. 2015; Riaz et al. 2019). Peng et al. (2016) reported that the mean concentrations of total 16 PAHs in suburban and rural soils of Beijing were 322 and 219 ng g^{-1} respectively, and the major sources of PAHs in these soils were coal and biomass combustion.

Besides traditional pollutants, contaminants of emerging concern (CECs), a diverse group of largely unregulated chemicals, have become another major concern for the scientific community and regulatory agencies (Du et al. 2014; Fairbairn et al. 2016). Because some of CECs exhibit toxic or endocrine disruption potential to biota at the relatively low concentration, such as di(2-ethylhexyl)phthalate, triclosan and propylparaben (Herrero et al. 2012), and others persist, could accumulate and eventually biomagnify in higher trophic level species (e.g., nonyphenol and fire retardants). Wu et al. (2014) reported that pharmaceutical and personal care products (PPCPs) were detected in 8 vegetables irrigated by reclaimed water without and with a fortification of PPCPs at 250 ng L^{-1}, and the concentrations in edible tissues were in the range of 0.01–3.87 and 0.15–7.3 ng g^{-1} (dry weight), respectively. CECs are continuously discharged into the environment from domestic and industrial sewage systems and affected by the level of urbanization and industrial point sources, even the concentration showed a clearly increasing pattern along the rural-suburban-urban gradient (Peng et al. 2016).

To date, the effects of TEs, PAHs, and CECs on peri-urban agriculture have been researched separately (Da Silva et al. 2017; Pan et al. 2014), but the knowledge about the co-influence of these compounds is scarce (Margenat et al. 2018; Marquès et al. 2017). When identifying this influence along with city activities on peri-urban soil, it is infeasible to test the toxicity of every chemical and their mixtures to all species. Thus, bio-monitoring strategies are becoming more used (Bagur-González et al. 2011; Li et al. 2017). As part of the battery of bioassays, seed germination and root elongation have been well developed and recommended by many regulatory agencies, because of the short experimental cycle and cost savings (Luo et al. 2018; Zaccheo et al. 2009). A previous study shows that germination was higher in seeds watered with irrigation waters than with distilled water, probably because the higher concentration of nutrients in the irrigation waters that would help break dormancy to facilitate seed germination (Margenat et al. 2017).

Given the facts mentioned above, this study aimed to develop a simple and rapid method to assess the effect of soil surroundings on peri-urban agriculture in the Baix Llobregat Agrarian Park (BLAP) in the city of Barcelona. To this end, we evaluated two plant growth indexes (seed germination rate and root elongation) under different soil environments (from peri-urban area and a pristine site). Here we present the different responses of three seeds: lettuce (*Lactuca sativa L.* cv. Quattro Stagioni), cauliflower (*Brassica cretica L.* cv. Maravilha), and tomato (*Solanum lycopersicum L.* cv. Marmande). They were selected because they represent three types of vegetables: leafy crop, inflorescence, and berry, which have been recommended by the US Food and Drug Administration and the Organization for Economic Cooperation and Development (OECD 2006) for bioassays and phytotoxicity tests. In addition, these three vegetables are popular in the Mediterranean diet.

Methods

Sampling site description

The study area (Fig. 1) is located in the delta and low valley of the Llobregat River (NE Spain) where adjoins Barcelona. In this region, based on the gradient of the impact of industrial, urban, and agricultural activities, three sites were chosen. Site 1 (S1) is an organic farming area irrigated by a drip system, manure-amended, relatively far away from Barcelona, and protected from urban pollution, while site 2 (S2) and site 3 (S3) are located in the peri-urban area and furrow irrigated with treated wastewater from the Llobregat River. Compared with S2, which has car traffic pollution, S3 is mainly influenced by the adjacent airport.

Sampling strategy

All soil samples were collected from the surface (0–20 cm depth) of three different study sites. A composite soil sample was obtained from five subsamples in each farm plot. Soil samples were sieved through a 2.0-mm mesh and stored at − 20 °C. According to the USDA (1987) classification, soil

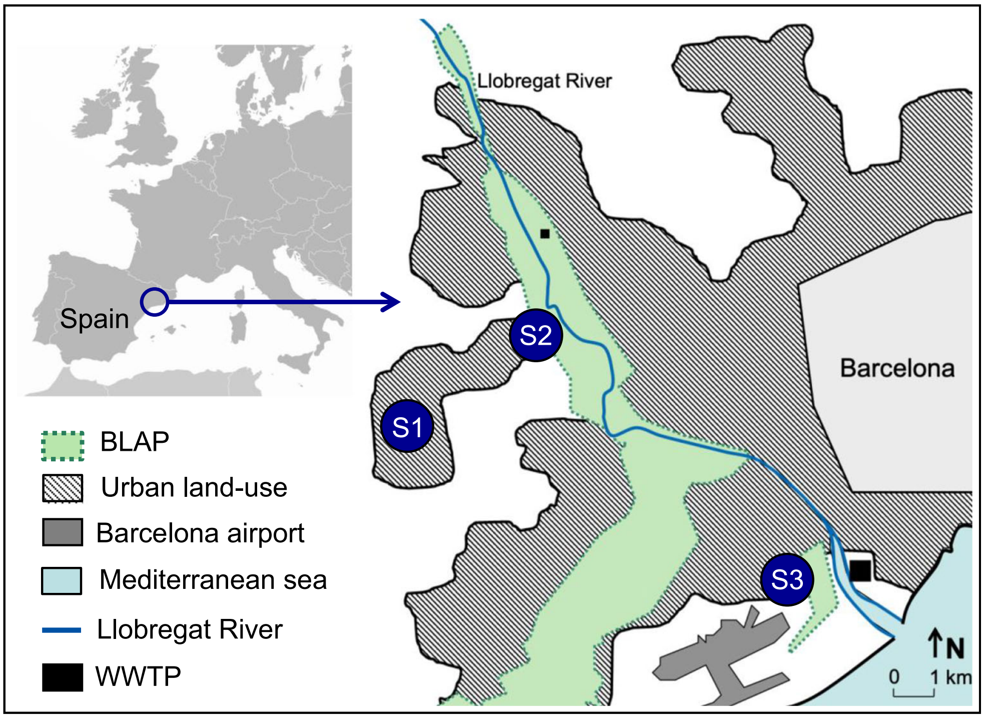

Fig. 1 Map of the agricultural area with sampling points. S1 is an organic farming area irrigated by a drip system; S2 and S3 are furrow irrigated with different treated wastewater from the Llobregat River

samples from farm plots S1 and S2 were loamy sand, while the soils from S3 was sandy.

Analysis procedure

Chemical analysis

Soil pH was determined in the suspension with a soil to water ratio of 1:2.5 using a pH meter, and electrical conductivity was measured in the saturation paste extract. Cation exchange capacity was determined by saturation with sodium acetate solution, replacement of the absorbed sodium with ammonium, and determination of displaced sodium by flame atomic absorption spectrometry. The concentration of $NaHCO_3$-extractable P was measured by the procedures developed by Olsen as described in Page et al. (1982). The soil samples were analyzed for nitrate as nitrate nitrogen (NO_3-N) by Hach-Lange spectrophotometer (DR 1900 Portable Spectrophotometer), and the NH_4Ac (CH_3COONH_4)-extractable speciation of K, Ca, Mg, and Na was determined by inductively coupled plasma optical emission spectrometry (Thermo Scientific, iCAP 6500 ICP-OES).

To determine the concentration of total trace elements, soils were analyzed by the method published by EPA (1994). Briefly, 1 g dried and homogenized soil was digested with 4 mL HNO_3 (concentrated HNO_3 to ASTM type I water ratio of 1:1, v:v) and 10 mL HCl (concentrated HCl to ASTM type I water ratio of 1:4, v:v) on a hot plate at 85 °C around 30 min, then transferred the mixture to 100 mL volumetric flask, and diluted to volume with reagent water. After centrifuging at 3756 × g for 20 min, 10 mL supernatant was diluted 5 times again, and the solution was measured using inductively coupled plasma mass spectrometry (Thermo Scientific, xSeries 2 ICP-MS).

According to the method published by Chamorro et al. (2013), the extraction of PAHs in soil was performed on a PSE-One (Applied Instruments, USA) by duplicate. Briefly, 3 g of freeze-dry sediment was extracted with n-hexane/acetone 1:1. The extraction conditions were 110 °C, 140 bar, and 3 cycles with solvent mixture of 15 min each. The cleanup of the extracts was carried by adsorption chromatography on a glass column packed with 5 g of neutral alumina (activated at 400 °C, deactivated with 3% water) and anhydrous sodium sulfate (top). Then, different fractions were eluted: FI (10 mL n-hexane), FII (10 mL n-hexane/ethyl acetate (9/1, v/v)), and FIII (10 mL n-hexane/ethyl acetate (1/1)). For PAH determination, FII was analyzed in a Bruker Scion 436GC SQ apparatus (Bruker Daltonics Inc., Billerica, MA, USA) using the following: splitless injection (hexane, 290 °C; the purge valve was activated 50 s after the injection); helium carrier gas (0.6 mL/min); a 20 m × 0.18 mm i.d. column with a 0.18μm coating of TRB-5MS stationary phase (from Teknokroma, Barcelona, Spain); a column temperature program consisting of 1.20 min at 60 °C, a 14 °C/min ramp up to 200 °C, a 7.5 °C/min ramp up to 300 °C, and 10 min at 300 °C; transfer line and ion source temperatures of 280 °C and 290 °C, respectively; data acquisition in full-scan mode from 50 to 500 amu, with 6.2 min of solvent delay; and data processing by Workstation 8 software.

Soil CEC extraction was adapted from a slightly modified method (Xu et al. 2008). Briefly, 5 g soil, homogenized and sieved through 2.0 mm mesh, was mixed with 5 mL acetone/ethyl acetate (1:1, v:v) solvent in 50 mL of screw Teflon lined cap centrifuge tube, sonicated at 42 kHz for 15 min, centrifuged at 8452 × g for 10 min, and decanted the supernatant. The soil was extracted three additional times with 4 mL, 5 mL, and 4 mL of solvent. The supernatants were combined and were nitrogen-evaporated in a water bath at 40 °C to about 1 mL. Then, the concentrated solution was re-dissolved in 500 mL de-ionized water, and the pH was adjusted to 3 by concentrated sulfuric acid. Then, the extraction was loaded onto previously conditioned SPE cartridges (STRATA X, 100 mg, 6 mL). The cartridge was eluted with 10 mL of ethyl acetate and was dried by nitrogen for 20 min to 250 μL. Besides, 37.25 ng of triphenylamine (TPhA) was added as an internal standard. Finally, a 50-μL aliquot was analyzed by GC–MS/MS without derivatization, and another 50 μL aliquot was analyzed derivatized with 10 μL of MTBSTFA.

The LODs and LOQs were calculated for each analyte as three and ten times the signal from the baseline noise (S/N ratio), respectively, and are provided in the tables. For calculation, levels below the LOQ and LOD were replaced by ½ LOQ and ½ LOD, respectively.

Seed germination and root elongation tests

The experiment was designed based on the guideline issued by Environmental Protection Agency (EPA 712-C-96-154) about seed germination/root elongation toxicity test and ISO 11269-1 root growth test. The seed species assayed were lettuce (*Lactuca sativa L.* cv. Quattro Stagioni), cauliflower (*Brassica cretica.* cv. Maravilha), and tomato (*Solanum lycoperscum.* cv. Marmande). Seeds for the experiments were purchased from a local garden material store. Before being tested for bioassays, their germination potential was examined under 24 ± 2 °C in the dark for 2 days. The germination rate for all tested seeds was over 90% which guaranteed the viability of the seed.

Soil was air-dried, mixed, and grounded to fine powder (< 2 mm). Then, 30 g of soil per site was weighed into a 100 × 15mm Petri dish and wetted with deionized water to reach 80% of its total water-holding capacity.

As for seeds, at first, they were sterilized with 2.5% sodium hypochlorite for 15 min and carefully washed with distilled water. Ten clean seeds were evenly put, at least 0.5 inches from the edge, on the surface of the soil per dish. Then, cover the dish to avoid moisture evaporation. Subsequently, all dishes were randomly placed on the lab workbench at 24 ± 2 °C in darkness for germination. According to the EPA guideline, germination means the resumption of active growth by an embryo. Moreover, as defined by Finkelstein et al. (2008), germination is the initial emergence of the radicle from the seed coat. The primary root should attain a length of 5 mm for the seed to be counted as having germinated. When at least 65% of seeds of control have germinated and developed roots that are at least 20 mm long, germination experiment concludes, and germination rate could be calculated. In our case, although tomato, lettuce, and brassica vary in their germination time in our control soil (30 to 45 h), all of them germinated within 48 h. Then, these seedlings were put under fluorescent lamp for 16:8 h light to dark cycles for the next 72 h for root to continue growing. Five replicates of each treatment were tested.

After the initial 48 h, in each dish, the number of acceptable seedlings was counted and divided by the total number of seeds added (10) to calculate the germination rate. At the end of the experiment (3 days later), all seedlings were pulled out and the root elongation was measured which is defined as the length from the tip to radicle.

Statistical analysis

The experimental data were statistically evaluated by SPSS v. 24 package (Chicago, IL, USA). One- or multi-way analysis of variance (ANOVA) and Pearson's correlation analysis were performed for multiple comparisons or for analyzing interactive effects between different factors. Principal component analysis (PCA) was conducted on the concentrations of TEs, PAHs, CECs, and agronomical indexes. Varimax rotation was applied because orthogonal rotation minimizes the number of variables with a high loading on each component and facilitates the interpretation of results. Statistical significance was defined as $p < 0.05$.

Result and discussion

Conventional soil quality parameters

Table 1 shows the soil characteristics from the different sites. The driest soil is from S3, which is significantly different from soils from S1 and S2 ($p < 0.05$), probably due to different soil textures. According to the USDA (1987) classification, the soil in S3 is sandy, while soils in S1 and S2 are loamy sand. It is well known that sandy soil with unstable structure has less capacity for holding soil water and fertilizer than sandy loam. Besides, high electrical conductive values were detected in all soil sites. Margenat et al. (2017) reported that in this area, treated wastewater has high conductivity, which may bring the high CE level in soil irrigated by it (S2, S3). As for S1, owing to long-term manure fertilization, the CE in soil inevitably increases. This is also consistent with the high values of N, P, and K.

Table 1 General quality parameters in the studied soils. Mean ± SD ($N = 3$). Different letters indicate significant differences among sites

	S1	S2	S3
Humidity 105 °C (%)	46 ± 1^a	64 ± 3^b	32 ± 2^c
pH	7.61 ± 0.01^a	7.74 ± 0.07^b	7.50 ± 0.40^c
Electrical conductivity (μS cm^{-1})	2331 ± 17^a	2198 ± 23^b	5010 ± 41^c
Nitrate (mg kg^{-1})	9.0 ± 0.7^a	2.0 ± 0.3^b	10.0 ± 1.1^c
Phosphorous (mg kg^{-1})	64 ± 4^a	35 ± 2^b	7 ± 3^c
Potassium (mg kg^{-1})	375 ± 21^a	346 ± 19^a	297 ± 9^b
Calcium (mg kg^{-1})	2984 ± 23^a	6561 ± 21^b	6422 ± 43^c
Magnesium (mg kg^{-1})	379 ± 22^a	360 ± 27^a	135 ± 13^b
Sodium (mg kg^{-1})	32 ± 2^a	136 ± 3^b	152 ± 7^c
Cation exchange capacity (ms cm^{-1})	7.5 ± 0.2^a	9.7 ± 0.7^b	8.0 ± 1.2^{ab}
Texture	Sandy loam	Sandy loam	Sandy

Occurrence of trace elements in soils

The concentration of TEs (16 out of the 58 elements analyzed) in the soils and the maximum acceptable values in agricultural soil established by the Catalan Law 5/2017 in accordance with the Spanish Royal Decree 9/2005 are listed in Table 2. The TEs detected at the highest value in all soils were Mn (361–494 mg kg^{-1}), Zn (132–217 mg kg^{-1}), and Ti (184–458 mg kg^{-1}). Mn and Zn as essential micronutrients participate in the structure of photosynthetic proteins and enzymes in plants and have a stimulating effect on auxin promoting coleoptile growth. However, once their concentrations in soil exceed the specific thresholds, they would also damage plants like other pollutants. In the peri-urban area, due to the high intensity of industrial activities, soil often contains high levels of Mn and Zn. In our studies, Mn and Zn levels in S2 and S3 soil are close or even above the regulated level values, and the result is consistent with the abundance of Mn (103–13,584 mg kg^{-1}) and Zn (23–3770 mg kg^{-1}) reported in soil from other Baix Llobregat sites (Zimakowska-Gnoińska et al. 2000). Therefore, it is imperative to control the sources of these two elements to avoid the negative effect, whereas the high abundance of Ti is consistent with geogenic origin. The content mainly depends on the parent rock and the degree of differentiation. S3 soil was mainly derived from shallow sea sediments that generally contain more Ti than the other two soil sites developed from granite (Boström et al. 1973).

Table 2 Concentrations of TEs in three sites and the limit value according to the Catalan soil law (mg kg^{-1} dw). Mean ± SD ($N = 3$). Different letters indicate significant differences among sites. Number in bold indicates that the value exceeds the limit

	S1	S2	S3	Threshold value
Ti	184 ± 15^a	260 ± 22^b	**458 ± 21^c**	390
V	46 ± 7^a	61 ± 5^a	71 ± 2^b	100
Cr	26 ± 2^a	66 ± 4^b	117 ± 12^c	400
Mn	361 ± 9^a	494 ± 18^b	471 ± 15^b	500
Co	7 ± 4^a	10 ± 5^a	10 ± 3^a	25
Ni	17 ± 3^a	34 ± 5^b	33 ± 5^b	45
Cu	34 ± 8^a	**179 ± 21^b**	91 ± 12^c	100
Zn	132 ± 7^a	**208 ± 11^b**	**217 ± 12^b**	200
As	16 ± 1^a	**38 ± 2^b**	26 ± 1^c	30
Ba	86 ± 3^a	305 ± 3^b	**510 ± 7^c**	500
Pb	16 ± 1^a	**164 ± 5^b**	**150 ± 8^b**	60
Cd	0.5 ± 0.1^a	0.7 ± 0.1^b	1.2 ± 0.1^b	2.5
B	92 ± 7^a	81 ± 8^a	101 ± 3^b	–
Li	11 ± 2^a	28 ± 1^b	28 ± 3^b	–
Mo	2.0 ± 0.2^a	2.0 ± 0.7^a	1.9 ± 0.4^a	3.5
Hg	0.01 ± 0.01^a	0.37 ± 0.01^b	0.60 ± 0.01^c	2

The total average concentration of TEs per site was as follows: 143 mg kg^{-1} (S3), 121 mg kg^{-1}(S2), and 64 mg kg^{-1}(S1). ANOVA test showed that soil from S1 was less contaminated by many trace elements (i.e., Cu, Pb, Zn) than either of soil in peri-urban (S2, S3, $p < 0.05$). In fact, according to the regional decree (Generalitat de Catalunya, 2017), the levels of all trace elements in S1 were lower than the threshold. In the S2 soil, the concentrations of Cu, Zn, As, and Pb exceed the standard guideline, while in the S3 soil, Zn, Pb, and Ti concentrations are higher than the norm. This fact may be explained by the different fertilization method, traffic pressure, and industrial inputs in each site. In S1, an organic amendment was used for soil fertilization, whereas in S2 and S3, inorganic fertilizer is commonly employed. Alloway (2012) reported that the application of phosphate fertilizer generally gives rise to the high concentration of most metal(loid)s such as As, Cd, and Zn. In addition, a large concentration of Pb was found in the S2 and S3 partly derived from legacy leaded automobile exhaust emissions in the urban area, and partly result from the irrigation water. Cabeza et al. (2012) reported that the value of Pb in irrigation water of studied area exceeded the maximum allowable concentration. Moreover, Nziguheba and Smolders (2008) found that phosphate fertilizers are the main source of increased Pb levels in cropped soils. High Zn levels in S2 and S3 are associated with traffic density since Zn sources include, among others, brake linings and rubber tires (Councell et al. 2004). Moreover, Zn has been used as a tire-wear particle tracer (Amato et al. 2011; Harrison et al. 2012).

Occurrence of PAHs in soils

Table 3 shows the 16 PAHs in soil samples and the maximum concentration of PAHs allowed by the Catalan legislation (Busquet 1997). Apparently, all of them are significantly below the limit values. The total concentrations of PAHs in detected soil were as follows: 24 ng g^{-1} dw (S1), 38.4 ng g^{-1} dw (S2), and 49 ng g^{-1} dw (S3), and they were in the same range as those in an uncontaminated area reported by Nadal (2004) in Catalonia. Based on these results, in Barcelona, the agriculture soil is not obviously polluted by PAHs. However, the contamination of PAHs is still positively related to the nearby urbanization level and traffic pressure (S3 > S2 > S1).

PAHs could be divided into two main classes: low and high molecular weight PAHs (LMW and HMW, respectively). The LMW PAHs (2–3 ring PAHs) such as naphthalene, fluorene, phenanthrene, and anthracene are shown to have significantly less toxicity compared to the HMW PAHs of 4–7 rings (from pyrene to indeno[1,2,3-c,d]pyrene in Table 3) which are recalcitrant and carcinogenic to humans (Duan et al. 2015; Kuppusamy et al. 2015). In our study, the concentrations of LMW were almost all below the limit of quantification. This may result from the high irradiation rate (mean 15 MJ m^{-2}, up to 25 MJ m^{-2} in July) in Barcelona (Meteocat 2019). It is well known that PAHs suffer degradation in the atmosphere by photo-oxidation (Balducci et al. 2017; Chao et al. 2019), especially for 2- and 3-ring PAHs which possess the low molecular weight, high vapor pressure, and high fugacity ratio (Mackay et al. 2000). In 1990, Park monitored that volatilization accounted for approximately 30 and 20% loss of naphthalene and 1-methylnaphthalene, respectively; but for the remaining compounds, volatilization was negligible. Kuppusamy et al. (2017) also reported that LMW PAHs would be lost rapidly and become more mobile and degradable once entering the soil, while HMW PAHs are more persistent and resistant to degradation (Kuppusamy et al. 2015). In addition, for HMW PAHs, due to relatively higher molecular weight, they tend to fall down to the soil near the emitted point by dry and wet deposition. Therefore, the concentrations of HMW PAHs in peri-urban soil (S2, S3) almost double that in the pristine site (S1). The total concentrations of HMW PAHs are low, but representative; therefore, only the total contamination of HMW PAHs will be involved in subsequent evaluations about phytotoxicity assessment, instead of individual PAH and all PAHs.

Occurrence of contaminants of emerging concern

A total of 33 CECs were analyzed; Table 4 shows only 11 of them which were detected above LOQs in at least one site. For different soils, the fluctuation of CEC concentration is different. S1 ranged from non-detectable to 397 ng g^{-1} (TCPP), S2 ranged from non-detectable to 6.68 ng g^{-1} (chlorpyrifos), and

Table 3 PAHs concentrations (ng g^{-1} dw) in three sites and the maximum concentrations allowed (data from the Catalan legislation for soil)

	S1	S2	S3	LOD	LOQ	Maximum value
Naphthalene	< LOD	< LOD	< LOQ	0.4	1.0	5000
Acenaphthene	< LOQ	< LOQ	< LOQ	0.1	0.1	–
Acenaphtnylene	< LOD	< LOQ	< LOQ	0.1	0.1	–
Fluorene	< LOD	< LOD	< LOQ	0.4	0.6	–
Phenanthrene	< LOQ	< LOD	< LOQ	1.1	2.3	5000
Anthracene	0.5	0.8	0.6	0.4	0.5	100000
Fluoranthene	2.0	< LOQ	2.3	0.9	1.7	15000
Pyrene	1.9	1.5	1.9	1.1	2.1	–
Benzo[a]anthracene	1.6	1.9	2.4	1.0	1.4	10000
Chrysene	2.3	2.1	2.4	0.9	1.4	–
Benzo[b]fluoranthene	2.9	4.2	4.8	1.0	1.6	–
Benzo[k]fluoranthene	< LOQ	4.1	5.0	1.5	3.6	50000
Benzo[a]pyrene	1.9	3.4	4.3	1.7	3.4	80
Dibenz[a,h]anthracene	1.1	1.9	2.0	0.9	0.9	–
Benzo[g,h,i]perylene	2.1	4.2	5.4	0.9	1.1	–
Indeno[1,2,3-c,d]pyrene	4.2	12.3	16.8	1.2	2.0	50000
∑16 PAHs	24	38.4	49			
∑ HMW PAHs	19.8	35.6	45			

Table 4 Concentrations of CECs (ng g^{-1} dw) in the different soils evaluated

	S1	S2	S3	LOD	LOQ
Azoxystrobin	nd	3.82	nd	0.36	0.37
Chlorpyrifos	nd	6.68	nd	0.04	0.06
N,*N*-Diethyl-meta-toluamide (DEET)	1.40	0.48	< LOQ	0.19	0.22
Tris(2-chloroethyl) phosphate (TCEP)	1.10	nd	nd	0.17	0.18
Bisphenol F (BPF)	199	< LOD	< LOD	9.0	10.1
Carbamazepine	< 0.12	0.14	< 0.12	0.12	0.14
Metylparaben (MPB)	< LOD	< LOD	30	6.18	6.92
1-Hydroxybenzotriazole (OHBT)	5.60	5.80	5.50	10.8	11.0
Pymetrozine	2.00	1.30	1.40	0.88	0.89
Carbamazepine-10,11-epoxide	nd	< LOD	< LOD	0.21	0.40
Tris (chloroisopropyl) phosphate (TCPP)	397	< LOD	< LOD	20.9	21.4

Concentration values have been corrected by the recoveries

nd not detected

S3 ranged from non-detectable to 30 ng g^{-1} (MPB), separately. It is interesting to note that the concentration of TCPP and BPF in S1 is obviously higher than S2 and S3. This phenomenon could be due to S1 unique irrigation system (dripping by plastic tubing) and organic fertilization method (manure). TCPP is an organic flame retardant used as a raw material in the manufacture of polyester, rubber, binder, and resins. In 2000, European production of TCPP reached 36,000 tonnes year^{-1} (Föllmann and Wober 2006), while Bisphenol F (BPF) is used in the production of epoxy resins and polycarbonate polymers for lining large food containers, water pipes, and mulch plastic film. Due to their chemical characteristics (TCPP: log K_{ow} = 2.98, water solubility = 1200 mg L^{-1}; BPF: log K_{ow} = 3.06, water solubility = 360 mg L^{-1}), both TCPP and BPF would release into the environment through the plastic tubing during irrigation. In addition, Fromme (2002) detected BPF in liquid manure (2.2–62.6 μg kg^{-1} dw). Overall, the concentrations of CECs in our studied soils were lower than other peri-urban areas (Mac Loughlin et al. 2017; Regueiro and Wenzl 2015).

Sensitivity of plant species

Effect of different soils on plant growth indexes

There were two endpoints measured, namely seed germination rate and root elongation (Table 5). The germination rates of lettuce and tomato could reflect the difference between three soil sites ($p < 0.05$), while cauliflower germination rate is relatively insensitive, only could separate S3 from S1 and S2. Furthermore, unlike lettuce and cauliflower of which the germination rate was as follows: S2 > S1 > S3, tomato germination rate in S2 (67%) is extremely lower than S1 (91%) and S3 (83%).

As for root elongation, lettuce also is a good bioindicator specie which grew significantly differently in different soils, S1 (36) > S2 (30) > S3 (21), whereas, for tomato and cauliflower, the lengths of root are not significantly different between S1 and S2 soil.

Relationships between soil parameters and plant growth indexes

Table 6 shows Pearson's correlation coefficients among soil parameters, seed germination rate, and the length of roots. It was found that for lettuce and cauliflower, their germination rates were significantly correlated with humidity and pH, while other factors were less significant (i.e., Mg, CEC, Mo) or had no correlation with them (i.e., Hg, Na, DEET), indicating that their seed germination is more sensitive to the moisture and pH rather than environmental pollution. Similar results were also reported by Rezvani et al. (2014). This may be explained by the following reasons. First, seed germination is simply an appearance of cell elongation instead of cell division (Haber and Luippold 1960), and it depends on the water

Table 5 Comparison of seed germination and root elongation for three kinds of vegetable in three studied sites

Site	Plants	Germination rate (%)	Root elongation (mm)
S1	Lettuce	94.0 ± 0.6^{a}	35.8 ± 3.6^{a}
	Tomato	91.4 ± 2.3^{a}	37.3 ± 9.4^{a}
	Cauliflower	89.4 ± 2.7^{a}	48.3 ± 7.9^{a}
S2	Lettuce	99.0 ± 0.9^{b}	30.2 ± 4.4^{b}
	Tomato	67.0 ± 4.3^{b}	38.3 ± 7.5^{a}
	Cauliflower	90.4 ± 1.2^{a}	50.6 ± 9.5^{a}
S3	Lettuce	91.0 ± 1.7^{c}	21.4 ± 3.2^{c}
	Tomato	83.0 ± 2.2^{c}	32.2 ± 4.7^{b}
	Cauliflower	80.2 ± 2.5^{b}	42.7 ± 8.1^{b}

For the same plant species, different letters indicate significant differences between soils ($p < 0.05$)

Table 6 Pearson correlation coefficients between the soil parameters and plant index

	Germination rate			Root elongation		
	Lettuce	Tomato	Cauliflower	Lettuce	Tomato	Cauliflower
Humidity	0.971	–	0.919	–	–	–
pH	0.959	–	0.930	–	–	–
EC	–	–	–	− 0.920	− 0.934	− 0.960
NO_3	–	0.988	–	0.991	0.978	–
K	–	–	–	1.000	0.988	–
Mg	–	–	–	0.958	0.966	0.946
Ti	–	–	–	− 0.996	− 0.990	–
V	–	–	–	− 0.947	–	–
Cr	–	–	–	− 1.000	− 0.986	–
Cu	–	− 0.977	–	–	–	–
As	–	− 0.981	–	–	–	–
Ba	–	–	–	− 0.983	− 0.960	–
Cd	–	–	–	− 0.998	− 0.991	–
Hg	–	–	–	− 0.962	− 0.939	–
MPB	–	–	–	− 0.936	− 0.947	− 0.956
HWM PAHs	–	–	–	− 0.958	− 0.936	–

All correlations shown are significant at the 0.01 level (2-tailed)

and the reserves in the seed itself instead of surrounding condition; therefore, the response to environmental pollution is not sensitive. Second, it is necessary to consider that the effect of contaminants on seed germination relies on their ability to reach embryo tissues across the physiological barriers, mainly, the seed coating. The contaminants occurring in the soil may be absorbed by the seed coating, thus would not affect the growth of the embryonic root.

The seed germination of tomato is obviously different from lettuce and cauliflower which has a significant negative relation with Cu and As and positively correlated with nitrate. It is consistent with the phenomenon reported by Ashagre et al. (2013) that increasing Cu concentrations to 100 mg kg^{-1} decreased significantly the tomato germination rate. This may be caused by (1) the key role of peroxidases which are Cu stress-related enzymes is regarded as stiffening cell wall. Peroxidase-catalyzed lignification decreases the cell wall plasticity and therefore reduces cell elongation (Sánchez et al. 1995). (2) Cu toxicity causes oxidative damage for seedling; Mazhoudi et al. (1997) observed the accumulation of lipid peroxidation products when the concentration of surrounding Cu increased. In addition, compared with lettuce

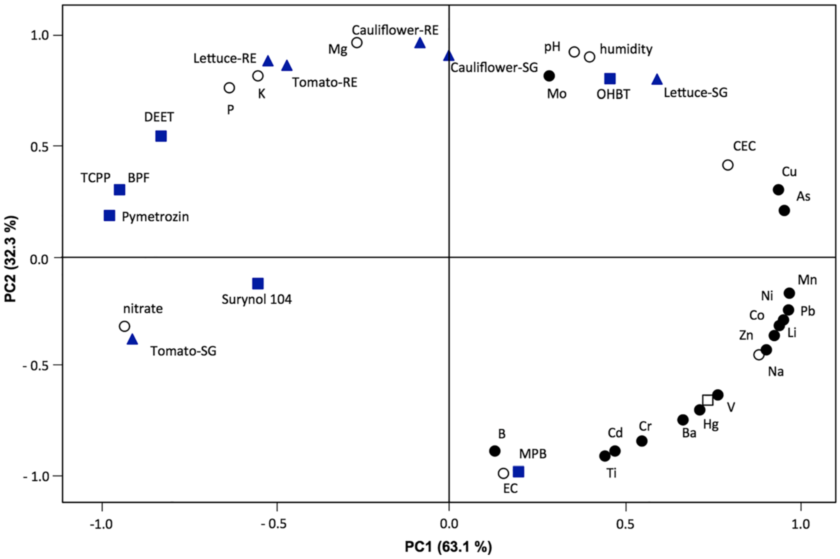

Fig. 2 Principal component analysis results, loading plot PC1 vs PC2. Trace elements: black circles; polycyclic aromatic hydrocarbons (PAHs): white square; contaminants of emerging concern: blue squares; conventional soil quality parameters and nutrients: open circles. Root elongation and seed germination: open triangles. RE: root elongation; SG: seed germination

and cauliflower, the germination of tomato seed seems to be also more sensitive to the excessive As and NO_3- in S2. In this sense, recently, Seifi et al. (2019) studied the effect of nitrate-reducing bacteria on the growth of tomato plants, since these bacteria have an important role in the biological removal of harmful nitrogen compounds. Some isolates increased significantly the growth parameters of tomato as well as seed germination, hypocotyl, and epicotyl length compared with control. Likewise, the existence of nitrate-reducing isolates in high nitrate surrounding may be the reason that tomato germination rate is higher in high NO_3- soil in our study.

On the other hand, the root elongation may be a better index to reflect soil condition due to the high correlation coefficients with more factors, especially with TEs and HMW PAHs. We will discuss it later based on PCA results.

Determination of the contributions of soil parameters to plant endpoints by PCA

Two principal components (PCs) extracted by PCA explained 63.1% and 32.3% of data variation (Fig. 2). The highest positive loading of PC1 was focused on TEs (Mn, Co, Ni, Cu, Zn, As, Pb, Li), indicating PC1 is mainly influenced by TEs. And the negative loadings of root length suggested that root elongation, especially lettuce root elongation, is a good index for the contamination of TEs, which is consistent with the results of Pearson's correlation analysis. Although the germination rate of tomato also has a high negative loading on PC1, it is not a perfect indicator for soil TEs, because as discussed before, it is only significantly related to pollution of Cu and As.

Except for tomato germination rate, all plant endpoints have high positive loading on PC2. For PC2, the highest positive loadings were humidity and pH which have been proved to be essential for the growth of seeds, and the highest negative loadings were Ti, Cd, B, EC, and OHBT, indicating these elements hinder the growth.

Figure 2 shows the loadings of PC1 versus PC2. The relationship between soil parameters and plant indexes is clearly observed. It was found that most TEs and PAHs were located in an adjacent area, focused on the fourth quadrant, especially concentrated on PC1, indicating that their variation may follow similar trends. All plant indexes in the second quadrant could reflect contamination, but their sensitivities are different. Among these indexes, cauliflower is less sensitive to pollution, due to the low loading on PC1. Excepted pollution, root length can suggest the soil fertility, since this index and nutrients (P, K, Mg) are located in the proximity area. Unexpectedly, CECs show a positive relationship with plant indexes. This may be because, in our study area, their concentrations are relatively low which may inhibit the growth of pathogens in the soil.

Conclusions

The results of this study show that risks posed to peri-urban agriculture ecosystem come from not only urban activities, but also from modern agricultural practices, so choosing proper plant can increase seed survival and improve yield. On the other hand, the feedback on the growth of seeds also could be an indicator of soil surrounding. In the present study, seed germination rate and root elongation were suitable for evaluating the chemical pollution of peri-urban agriculture soil of Barcelona. Along with the advantage of sensitivity of plant indexes, short experiment period (5 days), and low cost, the described method is useful when assessing peri-urban agriculture soils. Several key conclusions can be drawn:

1) The concentrations of TEs and PAHs in the soil from peri-urban agriculture area are below the guidelines' threshold, except for Cu, Zn, and Pb that exceed the limits.
2) Seed germination mostly depends on the humidity and pH. So compared with it, seed elongation is a better index which could reflect more aspects of the surrounding.
3) Low-dose CECs promote the growth of seedling.
4) Seed elongation could obviously show the pollution of TEs and PAHs in soil. Lettuce is the best indicator for assessing soil, due to the high seed germination and the significant decrease facing contamination.
5) It seems that tomato plants are not suitable for planting in the area with the highest Cu concentration, since it would significantly affect the seed germination rate, whereas cauliflower is less sensitive to pollution, therefore could be planted in the relative contaminated area.

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