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Lead in wild edible mushroom species in Leicester, England.

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Abstract: The aim was two-fold: to characterise the risks to lead (Pb) in *Agaricus bitorquis* collected in Leicester city (England); to evaluate its presence in urban topsoils. Pb was monitored by ICP-MS in twenty-two homogenised mushroom samples (caps and stipes) mineralised with HNO_3/H_2O_2 [LoD=0.872 mg/kg dry weight (dw)]. Moreover, 450 topsoil samples were collected from 18 urban parks across Leicester; Pb was also measured by ICP-MS after appropriate digestion (LoD=0.698 mg/kg). Levels were significantly higher in the mushroom caps (p-value=3E-05), median and ranges are provided in mg/kg dw: 2.461 (1.806-6.664) vs. 1.579 (0.988-4.223). Concentrations were much higher than those reported in sixteen *A. bisporus* (median <1.0 mg/kg DW) specifically cultivated in high traffic areas in the inner city of Berlin, suggesting some contamination by Pb. All caps monitored exceeded the established maximum concentration limit for Pb in cultivated mushrooms in the European Union (3 mg/kg dw), in line with the high accumulative metal capability described in the literature for *Agaricus* spp. Although non-carcinogenic risks characterised for Pb were negligible in the monitored mushrooms, high consumption of wild green edibles in Leicester's city should be limited as there are multiple additional sources of Pb and other metals, and substituted by cultivated edibles where possible.

Keywords: wild edible mushrooms; *Agaricus bitorquis*; Pb; risks; urban contamination.

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1. Introduction

Consumption of urban garden products, including wild edible mushrooms, can contribute to local food security, and is increasing all over the world despite the risks that they can represent due to anthropogenic contamination [1,2]. There is comprehensive evidence highlighting that urban and garden topsoils can contain high amounts of metals and metalloids. Wild mushrooms can grow in these areas, including highly polluted substrates, and can show a dynamic accumulation of trace metals, which would require careful assessing owing to the non-biodegradable nature of these pollutants [2]. Although the European Union has established EU standards for maximum acceptable metal concentrations in soils to be used for horticulture [3], collecting wild edible mushrooms can represent a serious risk for human health. Thus, recent systematic reviews have reported a significant toxic risk to those that consume wild edible mushrooms, including adults and children [4], highlighting a potential public health risk in England that has been little explored.

Evidence of the impact of urbanisation, economic development and growth on wild edible mushrooms in the British urban media is scarce. As a result, the aim was to assess the risks from lead (Pb) present in wild edible mushrooms of the species *Agaricus bitorquis* collected in Leicester city (England), as well as in urban topsoils, to evaluate the environmental presence and distribution of this contaminant.

2. Materials and methods

Twenty-two *A. bitorquis* mushrooms were collected from an open green area close to St Augustine Road, a high traffic area within the inner Leicester city centre. Mushrooms were manually washed with tap water to remove soil and dust, rinsed with deionised and Milli-Q water and split into caps and stipes to gain insight into the variability of Pb concentrations along the mushroom tissue [5]. Then, mushrooms samples were individually oven dried at 70-80 °C for 1 day [6]. The dried mushrooms' samples were ground into fine powder using a ceramic mortar [7], sieved through a 200-mesh sieve and finally homogenised and stored in polyethylene bags. Species identification was confirmed by DNA barcoding using internal transcribed spacer 1/4 primers after extracting DNA from 100 mg of frozen homogenised ground mushroom material using DNeasy Plant Mini Kit® (Qiagen Inc., Germantown, MD, United States), according to previous methods described by Sgamma et al. [8]. Pb was monitored by ICP-MS in cleaned, dried and homogenised mushroom caps and stipes mineralised with HNO_3/H_2O_2 [LoD=0.872 mg/kg dry weight (dw)], following previous methods [1].

Moreover, 450 topsoil samples were collected from 18 urban parks and green areas across Leicester, which were appropriately prepared, pulverised, pooled together and thoroughly homogenised on a motorised rotating mixer to be further processed as composite samples per park in duplicate. Pb was measured in duplicate in each of the 36 composite sample also by ICP-MS after acid digestion with nitric acid (69%) and chlorhydric acid (37%) in a microwave system [9].

Certified reference material for mushrooms (NIST1570a trace elements in spinach leaves; Sigma-Aldrich) and topsoils (CRM059 trace elements in loamy clay 2; Sigma-Aldrich) were used to assess quality of the measurements.

Non-carcinogenic human risks were assessed for exposure to Pb through ingestion, dermal absorption and inhalation of topsoils, following the US EPA Risk Assessment Guidance for Superfund (RAGs) methodology [10] and the dosimetry methodology [11]. Further equations and information can be found in Rovira et al. [12,13].

Statistical analysis Analyses were performed using the free software R, version 3.3.2. Peto-Prentice test was used to evaluate the differences between areas and to investigate tissue distribution. The levels of significance for statistical analyses were set at 0.05.

3. Results and discussion

Pb was found in all topsoil composites examined (LoD=0.698 mg/kg) and in the collected mushrooms. Percentages of recovery for the reference materials used for mushrooms and topsoils were 107% and 120%, respectively, indicating that the data obtained is accurate.

Levels of this metal were significantly higher in the mushroom caps (p-value=3E-05), median and ranges are provided in mg/kg dw: 2.461 (1.806-6.664) vs. 1.579 (0.988-4.223). Concentrations were in general lower to those recently reported in different species of the genus *Agaricus* collected in urban habitats within Berlin (Germany; <0.1-51.0 mg/kg dw) [1]. However, the levels of Pb detected were much higher than those reported in sixteen *A. bisporus* (median <1.0 mg/kg dw) specifically cultivated in high traffic areas in the inner city of Berlin reported by these authors, suggesting that Leicester's St Augustine Road presents a heavy volume of traffic that should be further explored to prevent risks to Pb exposure. Despite the ban of alkyl Pb motor fuel additives at the end of 1999 [14], road and traffic continue to be finite sources of Pb into the environment, which could include road runoff, corrosion of crash barriers, wear of tyres and engine pieces [15]. Moreover, diesel, biodiesel and unleaded gasoline can have ultra-trace amounts of Pb [16], which could contribute to the presence of this metal in the monitored environment owing to its high environmental persistence. Thus, Resongles et al. [14] has recently observed a strong link between the Pb deposited during the 20th century in London with its high presence in particulate matter in the current atmosphere of the city.

Higher levels of Pb were found in the urban area [102.805 (84.335-110.625) vs 62.080 (35.781-68.140); data presented as median and 95% CI, in mg/kg], which did not show statistical differences, perhaps due to the wide dispersion observed, which is characteristic of Pb and is well described in the literature. Leicester soils are less contaminated by Pb than other larger British cities, such as London (180.1 mg/kg) [17]. The urban region was subdivided in the four ordinal directions to study the distribution of this metal. Significant differences (P-value=0.02) were detected, which revealed the following pattern NW>SE>SW>NE for Pb, reflecting the wide distribution of this metal in Leicester.

3.1. Human health risks

All caps monitored exceeded the established maximum concentration limit for Pb in cultivated mushrooms in the European Union (0.3 mg/kg wet weight, approximately 3 mg/kg dw) [18], in line with the high accumulative metal capability described in the literature for *Agaricus* spp. [1]. However, the consumption of the monitored wild mushrooms represents a minimal risk to Pb, as the hazard quotients were much lower than the established threshold in both adults (5.72E-08) and children (2.67E-07).

Moreover, the levels of Pb detected in Leicester urban topsoils would not represent a toxic risk for the population for any route (oral, inhalation and dermal), as all the risk quotients (4.17E-02, 1.50E-04, 2.03E-01) were lower than the established threshold (unit). However, although the concentration range of Pb observed was similar to the England ambient background concentration [29.30-476.92 vs 40.1-387 mg/kg] [19], some areas within Leicester city could require remediation, as the levels of this metal exceed the upper range of the provisional Category 4 Screening Levels (pC4SLs) of Pb of residential land-use (130-330 mg/kg) [17,20].

4. Conclusions

Although non-carcinogenic risks characterised for Pb were negligible in the monitored mushroom, high consumption of wild green edibles in Leicester's city should be limited as there are multiple additional sources of Pb and other metals, and substituted by cultivated edibles when possible.

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