



# Lead in Wild Edible Mushroom Species in Leicester, England <sup>†</sup>

Gurminderjeet S. Jagdev <sup>1</sup>, Mark D. Evans <sup>1</sup>, Tiziana Sgamma <sup>1</sup> , María del Carmen Lobo-Bedmar <sup>2</sup> and Antonio Peña-Fernández <sup>1,3,\*</sup>

<sup>1</sup> Leicester School of Allied Health Sciences, Faculty of Health & Life Sciences, De Montfort University, Leicester LE1 9BH, UK

<sup>2</sup> Departamento de Investigación Agroambiental, Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Finca el Encín, Ctra. Madrid-Barcelona, Km 38.2, 28800 Alcalá de Henares, Spain

<sup>3</sup> Department of Surgery, Medical and Social Sciences, Faculty of Medicine and Health Sciences, University of Alcalá, Ctra. Madrid-Barcelona, Km. 33.600, 28805 Alcalá de Henares, Spain

\* Correspondence: antonio.penafer@uah.es

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**Abstract:** The aim was two-fold: to characterise the risks of lead (Pb) in *Agaricus bitorquis* collected in the city of Leicester (England), and 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<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> [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 =  $3 \times 10^{-5}$ ); 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, a 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 they should be substituted by cultivated edibles where possible.

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



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## 1. Introduction

The 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 assessment 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 to 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 the city of Leicester (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, mushroom samples were individually oven dried at 70–80 °C for 1 day [6]. The dried mushrooms' samples were ground into a 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 a DNeasy Plant Mini Kit® (Qiagen Inc., Germantown, MD, USA), 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 were mineralised with HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> [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 also measured in duplicate in each of the 36 composite samples by ICP-MS after acid digestion with nitric acid (69%) and chlorohydric acid (37%) in a microwave system [9].

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

Non-carcinogenic human risks were assessed for exposure to Pb through the 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 analyses were performed using the free software R, version 3.3.2. A 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 were accurate.

Levels of this metal were significantly higher in the mushroom caps ( $p$ -value =  $3 \times 10^{-5}$ ); 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 than those recently reported in different species of the genus *Agaricus*, collected in urban habitats in 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 of 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 in the environment, which could include road runoff, corrosion of crash barriers, and the 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] have 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 in other, larger British cities, such as London (180.1 mg/kg) [17]. The urban region was subdivided into four ordinal directions to study the distribution of this metal. Significant differences ( $p$ -value = 0.02) were detected, which revealed the following pattern for Pb: NW > SE > SW > NE, reflecting the wide distribution of this metal in Leicester.

#### *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 range of Pb found was lower than the maximum concentration limit established for wild fungi by the European Commission (0.8 mg/kg wet weight, about 8 mg/kg dw) [18]. Moreover, 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 ( $5.72 \times 10^{-8}$ ) and children ( $2.67 \times 10^{-7}$ ).

In addition to this, the levels of Pb detected in Leicester urban topsoils would not represent a toxic risk to the population for any route (oral, inhalation and dermal), as all the risk quotients ( $4.17 \times 10^{-2}$ ,  $1.50 \times 10^{-4}$ ,  $2.03 \times 10^{-1}$ ) 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 the city of Leicester 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, the high consumption of wild green edibles in Leicester's city centre should be limited as there are multiple additional sources of Pb and other metals, and they should be substituted by cultivated edibles when possible.

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