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Proceeding Paper

Risk to Humans Posed by Vanadium from Naturally Growing Edible Mushrooms and Topsoils across Leicestershire, UK †

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Abstract: The aims were: (a) to biomonitor vanadium (V) in wild edible mushrooms collected from urban/rural areas across Leicestershire (England); (b) to characterise the risk to humans caused by its content in topsoils. Thirty-four mushrooms were collected: twenty-two *Agaricus bitorquis* from a green area close to a busy traffic area; four *Marasmius oreades* from the NE; and eight *Coprinus atramentarius* from Bradgate Park, which is northwest of Leicester city. Moreover, 850 topsoil samples were collected and processed as composite samples (eighteen urban and eight rural), which were further processed in duplicate. The level of V was measured twice in each of the 52 composite samples and in the cleaned, dried and homogenised mushrooms by ICP-MS. Significant higher levels of V were found in *C. atramentarius* (0.856 (0.175–4.338)) than in the edible mushrooms collected in the urban areas (*M. oreades*, 0.305 (<LoD-0.852), and *A. bitorquis*, 0.078 (<LoD-0.187); the median and range are given in mg/kg dw). The health risk quotients calculated suggested a minimal risk to V if eaten. The presence of V in Leicestershire's topsoils would also not represent a significant risk for the population.

Keywords: vanadium; wild edible mushrooms; topsoils; food risks; human risks; Leicestershire

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1. Introduction

Vanadium (V) is the 21st most abundant element in the Earth's crust, water bodies and atmosphere [1]. Humans are exposed to small amounts of V through their diet and drinking water; although it does not represent a potential risk, as most of the dietary V is excreted in faeces [2]. V plays an essential and toxic role in humans at concentrations below 10 µg/day; meanwhile, at concentrations higher than 3 mg/day, it is considered extremely toxic for humans [3]. Contrarily, the essentiality of V has been proven for some terrestrial fungi [4]. For example, some wild mushrooms can accumulate high amounts of this element, such as the poisonous species of *Amanita* [4,5]. Although the presence of V in mushrooms could represent a risk for human health, the data on the mineral profile of V in cultured and wild edible mushrooms are still limited. This information is crucial to protect human health, as metal accumulation in mushrooms can significantly vary between species, including within the same taxonomic rank [6]. Thus, Neves et al. [3] has reported on the concentrations of V in shitake mushrooms that are below the limit of detection (0.022 µg/kg), while Niedzielski et al. [6] has described averages of V of 1.3 mg/kg dry weight (dw) in *Lactarius deliciosus*.

Owing to the lack of data regarding the current presence and distribution of V in the English urban media, the aims of our work were: (a) to biomonitor V in wild edible

mushrooms collected from urban and rural green areas across Leicester, a main city in East Midlands (England); and (b) to determine the presence and distribution of V in topsoils to identify the potential risks to humans.

2. Material and Methods

A total of 34 edible mushrooms were collected as follows: twenty-two *Agaricus bitorquis* mushrooms were collected from an open green area close to St Augustine Road, a busy traffic area close to Leicester city centre; four *Marasmius oreades* from Jesse Jackson Park in the NE of the city; and eight *Coprinopsis atramentaria* (previously known as *Coprinus atramentarius*) from Bradgate Park, an 850 acre public park in Charnwood Forest, northwest of Leicester city. Although *C. atramentarius* has been described as poisonous, it is generally considered edible and safe if cooked and if no alcohol is ingested within 2–3 days of eating the mushroom [7,8] because these species can produce coprine, especially when they mature, which is a disulfiram-like compound that reacts with alcohol and produce a toxic reaction, including vomiting [9].

The mushroom samples were carefully cleaned, dried and homogenised as previously described by our team in Jagdev et al. ([10]; *submitted for publication*). Briefly, species identification was confirmed by DNA barcoding using internal transcribed spacer 1/4 primers after extracting the DNA from 100 mg of frozen homogenised ground mushroom material using the DNeasy Plant Mini Kit® (Qiagen Inc., Germantown, MD, USA) following the methods described by Sgamma et al. [11]. V was monitored by ICP-MS in the homogenised mushrooms mineralised with HNO_3/H_2O_2 [10].

Additionally, 850 topsoil samples were collected across 18 different urban parks and open green areas across Leicester city and 8 surrounding rural areas from 2017 to 2018, which were appropriately prepared, pulverised, pooled together and thoroughly homogenised with a motorised rotating mixer to be further processed as composite samples per park in duplicate. V 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%) with a microwave [12].

The quality of the measurements was checked using the certified reference materials (Sigma-Aldrich, Saint Louis, MO, USA) of NIST1570a and CRM059 for mushrooms and topsoils, respectively.

Human health risk tests were carried out following the US EPA Risk Assessment Guidance for Superfund (RAGs) methodology [13,14], which have been described in more detailed by Peña-Fernández et al. [15].

Statistical analyses were performed using the free software R, version 3.3.2. The data were processed with the 'NADA' statistical package available in R, owing to the high levels of censored data for V found in the mushroom samples analysed (38%; LoD = 0.062 mg/kg dw). Thus, the data were processed following the recommendations suggested by Shoari and Dubé [16]. The Peto-Prentice test was used to identify differences in the concentrations between areas. The levels of significance for statistical analyses were set at 0.05.

3. Results and Discussion

The recoveries recorded for the reference materials used suggest that the methods used were appropriate (80% and 104% for NIST1570a and CRM059, respectively) [17].

Significantly higher levels of V were found in *C. atramentarius* (0.856 (0.175–4.338)) than in the edible mushrooms collected in the urban areas (*M. oreades* 0.305 (<LoD-0.852) and *A. bitorquis* 0.078 (<LoD-0.187); all the data are presented as median and range in mg/kg dw; p-value = 2×10^{-7}). The levels of V were similar to those reported in twelve different edible mushroom species collected in Finland (range reported 0.04–0.33 mg/kg dw; [18]), although very high levels of V were found in some of the eight *C. atramentarius* mushrooms collected. These authors also reported an average of 0.07 mg/kg dw of V in the *Boletus* species, which are very similar to those detected in *A. bitorquis* collected in Leicester city centre.

These results might suggest that, in general, Leicester city supports the low-level contamination of V. Thus, the health risk quotients calculated for the consumption of the edible mushrooms monitored (7.06×10^{-10}, 2.76×10^{-9}, 7.74×10^{-9} for adults and 3.29×10^{-9}, 1.29×10^{-8}, 3.61×10^{-8} for children; for *A. bitorquis*, *M. oreades* and *C. atramentarius*, respectively) are below the safety threshold, suggesting the minimal risk posed by V for these individuals (adults and children) that have incorporated these species collected in Leicestershire in their diets.

In relation to the environmental presence of V, significantly higher levels were found in the rural areas [61.403 (36.029–99.806) vs. 46.279 (25.861–84.653); the data are presented as the median and range in mg/kg; p-value = 3×10^{-5}], which might be attributed to geogenic sources as the enrichment factor for rural topsoils, suggesting minimal enrichment (0.0046–1.690). Despite this minimal enrichment, the presence of V in Leicestershire does not represent a significant risk for the population, as the hazard quotients for oral, dermal and inhalation exposure to V in the topsoils calculated were much lower than the threshold considered as safe for adults and children.

4. Conclusions

In general, although the risk posed by V present in wild edible mushroom species collected across Leicestershire would be minimal for adults and children, these species should be avoided owing to the fact that V can bioaccumulate in the bones and other human tissues, presenting a potential summative effect in conjunction with other toxic or carcinogenic metals and metalloids that could be present in these edible mushrooms. Moreover, the incorporation of wild species or *C. atramentarius* mushrooms into people's diets should be also avoided owing to their potential risks to human health, even though if these mushroom species are properly cooked and intake of alcohol is avoided.

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