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**Mercury in Bay Bolete (*Xerocomus badius*):
bioconcentration by fungus
and assessment of element intake by humans eating
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Food Types:	Mushrooms
Abstract:	Concentrations of Hg were determined in 221 specimens of Bay Bolete and in 221 samples of corresponding forest topsoil layer (0-10 cm) collected from 18 sites across geographically and industrially diverse regions of

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	<p>Poland in 2000-2008. Mercury concentrations in caps and stipes of Bay Bolete were strongly correlated ($p < 0.0001$), whereas the relationship between the Hg concentrations in soil and mushrooms varied depending on the sampling location. The Bay Bolete showed a lower bioconcentration potential of Hg at sites with elevated soil Hg concentrations and a higher at sites with lower Hg concentrations in soil. In a view of Hg content, the consumption of Bay Boletes (caps or a whole mushrooms) at the regions surveyed at least at a rate up to 2.5 kg per capita weekly in a mushrooming season is safe and will not result in exceeding of currently allowable Hg intake doses.</p>

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3 **1 Mercury in Bay Bolete (*Xerocomus badius*): bioconcentration by fungus**
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6 **2 and assessment of element intake by humans eating fruiting bodies**
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19 Abstract

20 Concentrations of mercury (Hg) were determined in 221 specimens of Bay Bolete and in 221
21 samples of corresponding forest topsoil layer (0-10 cm) collected from 18 sites across
22 geographically and industrially diverse regions of Poland in 2000-2008. Mercury concentrations in
23 caps and stipes of Bay Bolete were strongly correlated ($p < 0.0001$), whereas the relationship
24 between the Hg concentrations in soil and mushrooms varied depending on the sampling location.
25 The Bay Bolete showed a lower bioconcentration potential of Hg at sites with elevated soil Hg
26 concentrations and a higher at sites with lower Hg concentrations in soil. In a view of Hg content,
27 the consumption of Bay Boletes (caps or a whole mushrooms) at the regions surveyed at least at a
28 rate up to 2.5 kg *per capita* weekly in a mushrooming season is safe and will not result in
29 exceeding of currently allowable Hg intake doses.

30
31 **Keywords:** food contamination; heavy metals; mushrooms; nutrition; mercury.

33 Introduction

34 Contamination of food resources with Hg from environmental releases of this hazardous metal is
35 a continuous threat to food safety (Olivero et al., 2002). In order to protect human exposure from
36 Hg found in the food supply, the Joint FAO/WHO Expert Committee on Food Additives
37 established for total Hg a new provisional tolerable weekly intake (PTWI) of 0.004 mg kg⁻¹ body
38 weight in 2010 (JECFA, 2010). A few earlier studies have shown that wild mushrooms can
39 accumulate mercury from the soil to a considerable amount (Alonso et al., 2000; Chudzyński et
40 al., 2009 and 2011; Falandysz et al., 2001a, 2002a-b, 2003a-d and 2004; Melgar et al., 2009;
41 Stijve and Cardinale, 1974; Zimmermannova et al., 2001). The Bay Bolete mushroom, due to its
42 size and seasonal abundance, is by volume, one of the dominant species of mushrooms harvested

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3 43 in wild each year in Poland. For example, 1705 tons of mushrooms have been harvested from the
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5 44 wild by commercial harvesters in Poland in 2000, and it was 5914 tons in 2008, of which 3275
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8 45 tons were solely of the genera *Xerocomus* (*X. badius*, *X. subtomentosus*, *X. chrysenteron*) (GUS,
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10 46 2009). These quantities do not account for the amount harvested annually by numerous non-
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12 47 commercial collectors in Poland, where wild mushroom picking is a traditional activity.

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15 48 Some species of higher fungi (macromycetes) are known for their ability to accumulate
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17 49 metallic elements including hazardous heavy metals such as Hg, Cd or Pb (Brzostowski et al.,
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19
20 50 2009 and 2011a-b; Chudzyński and Falandysz, 2008; Doğan et al., 2006; Falandysz et al., 2001b,
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22 51 2007a-c, 2008a-b and 2011; Frankowska et al., 2010; Gast et al., 1988; Gucia et al., 2011a-b;
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24 52 Jarzyńska et al., 2011; Kojta et al., 2011; Malinowska et al., 2004; Melgar et al., 1998; Stijve and
25
26 53 Besson, 1976). Concentrations of trace elements in underlying soil or other substratum and
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28 54 bioavailability are among the factors that can affect the concentrations of metals in fruiting
29
30 55 bodies (carpophores) of fungi (Bargagli and Baldi, 1984; Borovička et al., 2010; Falandysz et al.,
31
32 56 1994; Řanda and Kučera, 2004; Stijve et al., 2004).

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36 57 Nevertheless, in nature, it is difficult to establish quantitative relationship between the
37
38 58 concentrations of metallic element and metalloid content in mushrooms and the underlying
39
40 59 substratum (Gast et al., 1988; Falandysz et al., 2011). If mushrooms and topsoil were collected
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42 60 concurrently from both contaminated and background (free of local pollution sources) areas, it is
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44 61 possible to elucidate the relationship soil metal concentrations and residue levels in mushrooms
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46 62 (Barcan et al., 1998; Falandysz et al., 2002c; Carvalho et al., 2005; Stijve and Roschnik, 1974).

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50 63 In background areas, Cd concentrations in fruiting bodies of the King Bolete (*Boletus edulis*)
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52 64 fungus increased with Cd concentrations in topsoil (Falandysz et al., 2011). A positive
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54 65 association was found between soil methyl mercury concentrations and the concentrations in
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56 66 fruiting bodies of fungi, although the number of evidences is very limited (Fischer et al., 1995). A
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3 67 similar relationship was also observed for total Hg in Parasol Mushroom (*Macrolepiota procera*)
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5 68 (Falandysz and Chwir, 1997).
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8 69 In this study, we determined total Hg concentrations in Bay Bolete (*X. badius*), and in
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10 70 forest topsoil layer below the fruit bodies, at eighteen sites distantly distributed across Poland.
11
12 71 We examined bioconcentration potential of Hg in Bay Bolete and daily intake rates of Hg from
13
14 72 mushroom consumption. Bay Bolete is a mycorrhizal fungus associated with coniferous
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16 73 woodland and mixed woodland. The symbiotic trees are supplied by fungus with portion of
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18 74 minerals absorbed by mycelium. Nevertheless, plants are very poor in Hg while mushrooms are
19
20 75 relatively abundant in Hg (Cibulka et al., 1996).
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25 76 **Materials and methods**

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27
28 77 Bay Bolete, *Xerocomus badius* (Fr.) Kühn, is a mycorrhizal mushroom and is a common
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30 78 species found in mixed woodlands of several European countries. The mushroom and topsoil
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32 79 samples (0-10 cm layer; ca. 100 g) were collected from eighteen, geographically diverse
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34 80 regions of Poland during 2000-2008 (Figure 1; Table 1). Mushrooms were cleaned up from
35
36 81 adhering plant material and soil particles with a plastic knife and brush and, if necessary, the
37
38 82 bottom part of stipe was cut away. Next, the fruiting bodies were initially air-dried for 48 h, and
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40 83 then were oven-dried at 65 °C to a constant weight. Dried mushrooms were crushed and ground
41
42 84 in a ceramic mortar to fine powder and kept in a sealed polyethylene bags. Soil substrate
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44 85 samples, after the removal of visible organisms, stones, sticks, and leaves, were air-dried for
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46 86 approximately 10 weeks and then sieved through a 2 mm sieve (Falandysz, 2002; Falandysz et
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48 87 al., 2003a).
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54 88 Analysis of total Hg content in fruiting bodies and soil was conducted using a direct
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56 89 sample thermal decomposition coupled with gold wool trap, desorption and cold-vapor atomic
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3 90 absorption spectroscopy (CV–AAS; Mercury analyzer type MA–2000, Nippon Instruments
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5 91 Corporation, Takatsuki, Japan) determination (Jarzyńska and Falandysz, 2011a). The procedure
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7 92 for Hg determination was validated through analysis of several certified reference materials
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9 93 including: CS-M-1 (dried fruiting bodies of mushroom, Cow Bolete *Suillus bovinus*); CTA-OTL-1
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11 94 (tobacco leaves) and INCT-TL-1 (tea leaves) produced by the Institute of Nuclear Chemistry and
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13 95 Technology in Warsaw, Poland. The declared total Hg contents of the certified reference materials
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15 96 were: $0.174 \pm 0.018 \mu\text{g g}^{-1}$ dw for CS-M-1 (our measurements were $0.179 \pm 0.008 \mu\text{g g}^{-1}$ dw; $n = 3$);
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17 97 $0.043 \mu\text{g g}^{-1}$ dw for CTA-OTL-1 (our measurements were $0.046 \pm 0.002 \mu\text{g g}^{-1}$ dw; $n = 3$); and
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19 98 $0.005 \pm 0.001 \mu\text{g g}^{-1}$ dw for INCT-TL-1 (our measurements were $0.005 \pm 0.000 \mu\text{g g}^{-1}$ dw; $n = 3$). In
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21 99 addition, with every set of 10 mushroom or soil samples analyzed, one procedural blank was
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23 100 included; no contamination or interference was found in blanks (Jarzyńska and Falandysz, 2011a).
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29 101 Data were evaluated statistically using the computer software Statistica version 8.0.
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31 102 Mercury concentrations in mushrooms and soils have no Gaussian distribution. Data
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33 103 transformation, which aimed to obtain their log-normal distribution, was unsuccessful. Moreover,
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35 104 data variances were heterogeneous (Barlett test). Consequently, statistical analyses were
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37 105 performed with nonparametric tests. A nonparametric Tukey-type multiple comparisons
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39 106 (Nemenyi test) following nonparametric analysis of variance (Kruskall-Wallis test) were applied
40
41 107 to indicate diversity of Hg content in soil substratum, and caps and stipes or whole fruiting bodies
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43 108 of specimens from the certain sampling sites surveyed. Further examination of differences in Hg
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45 109 concentrations between the caps (also stipes and soil) from certain localizations was made using
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47 110 Mann–Whitney U test.
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53 112 **Results and discussion**

54 113 ***Hg in fruiting bodies***

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3 114 Concentrations of total Hg in caps and stipes of Bay Bolete were high in specimens collected
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5 115 from Złotoryja (site 12 Z), and these concentrations differed significantly from samples collected
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8 116 at other locations (Figure 3). The arithmetic mean concentrations of Hg in Bay Bolete's cap
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10 117 varied between 0.051 ± 0.030 and $1.0 \pm 1.1 \mu\text{g g}^{-1}$ dw, depending on the location. The
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12 118 corresponding concentrations of Hg in stipes of Bay Bolete at these sites were between
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15 119 0.027 ± 0.018 and $0.68 \pm 0.86 \mu\text{g Hg g}^{-1}$ dw. The whole fruiting bodies collected from Kłodzka
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17 120 Dale (14 KD) contained $0.26 \pm 0.06 \mu\text{g Hg g}^{-1}$ dw. A total Hg concentration of $1.0 \mu\text{g g}^{-1}$ dw (0.1
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19 121 $\mu\text{g g}^{-1}$ wet weight) exceeded in two specimens from Włoszczowa (15 W) and two from Złotoryja
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21 122 (12 Z) (Table 1).
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24 123 Mercury concentrations in Bay Bolete at sites that are considered as background
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26 124 (unpolluted) areas varied between 0.051 ± 0.030 and $0.32 \pm 0.17 \mu\text{g g}^{-1}$ dw in caps and between
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28 125 0.027 ± 0.018 and $0.23 \pm 0.12 \mu\text{g g}^{-1}$ dw in stipes (Table 1). These concentrations are relatively
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30 126 small and comparable to a range of values between 0.26 ± 0.17 and $0.50 \pm 0.10 \mu\text{g g}^{-1}$ dw in caps,
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32 127 and between 0.089 ± 0.026 and $0.16 \pm 0.07 \mu\text{g g}^{-1}$ dw in stipes, reported for the mushroom Larch
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34 128 Bolete (*Suillus grevillei*) as well as between 0.095 ± 0.082 and $0.28 \pm 0.07 \mu\text{g g}^{-1}$ dw in caps and
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36 129 between 0.045 ± 0.018 and $0.13 \pm 0.02 \mu\text{g g}^{-1}$ dw in stipes reported for Slippery Jack (*Suillus*
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38 130 *luteus*) in Poland (Chudzyński et al., 2009 and 2011). The fungus, Bay Bolete, can be considered
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40 131 as a weak accumulator of Hg in fruiting bodies in comparison with King Bolete (*Boletus edulis*)
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42 132 which contained between 1.2 ± 1.4 and $7.6 \pm 3.1 \mu\text{g Hg g}^{-1}$ dw in caps or with Parasol Mushroom
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44 133 (*Macrolepiota procera*) which contained between 1.1 ± 1.0 and $8.4 \pm 7.4 \mu\text{g Hg g}^{-1}$ dw in caps
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46 134 (Falandysz and Gučia, 2008; Falandysz et al., 2007b and 2008a).
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53 135 The caps of Bay Bolete, on average, contained higher concentrations of Hg than the stipes
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55 136 (Table 1). The median value for caps to stipe Hg concentration quotient (Q_{CS}), calculated for 221
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57 137 specimens collected across Poland, was 1.7, and the arithmetic mean was 1.8 ± 0.8 (0.1-6.6).
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3 138 Nevertheless, the total Hg content in caps of Bay Bolete was significantly correlated with the
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5 139 content in stipes ($r = 0.95$; $p < 0.0001$; Figure 2).
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8 ***Hg content in topsoil and its relation to concentrations in mushroom***

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10 141 Soil Hg content is an important factor that affects the concentrations of this element in terrestrial
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12 142 plants and edible mushrooms. A concentration of Hg of $1.0 \mu\text{g g}^{-1}$ dw has been suggested as a
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14 143 reference value for agricultural soil (Rundgren et al., 1992). No such value has been reported for
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16 144 forest soils in Poland. The median Hg concentrations in topsoil at most sampling sites in this
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18 145 study were below $0.1 \mu\text{g g}^{-1}$ (Table 1). In our earlier surveys, we found that the median values of
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20 146 total Hg concentrations in forest soils in central and northern Europe were below $0.1 \mu\text{g g}^{-1}$
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22 147 (Chudzyński et al., 2009 and 2011; Falandysz and Bielawski, 2001 and 2007; Falandysz and
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24 148 Brzostowski, 2007; Falandysz and Chwir, 1997; Falandysz et al., 2001a, 2002a-c, 2003a-d and
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26 149 2004). It is known that the forest upper layer of soil that is rich in organic matter can easily
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28 150 absorb Hg deposited from the atmosphere and from weathering of parent bedrocks (Suchara and
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30 151 Sucharová, 2002). Nevertheless, the content of organic matter in the top 0-10 cm forest soil layer
31
32 152 vary greatly depending on the region, bedrock, vegetation, and age of the forest. The Critical
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34 153 Limit (CL) proposed for Hg(II) in soil is $0.13 \mu\text{g g}^{-1}$ (when soil properties are taken into account,
35
36 154 the value ranges between 0.04 and $1.2 \mu\text{g g}^{-1}$). The soil organic matter (SOM) normalized CL is
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38 155 $3.3 \mu\text{g Hg g}^{-1}$ of SOM (varies between 0.03 and $3.3 \mu\text{g g}^{-1}$). This CL value is greater than the
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40 156 value of $0.5 \mu\text{g g}^{-1}$ SOM, used to estimate Hg effects in European soil ecosystems (Tipping et al.,
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42 157 2010). The total Hg content in forest topsoil (0-10 cm layer) in this study, and in our earlier
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44 158 studies across Poland, was well below the recently proposed CL value for Hg(II) of $0.13 \mu\text{g g}^{-1}$.
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54 159 In earlier two studies, the concentrations of Hg in forest soils were found not dependent
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56 160 on soil type and texture or were elevated in soils of loose sands and slightly clayey relatively rich
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3 161 in humus type (Falandysz et al., 2002b and 2003a). It is worth to mention that no relation with Hg
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5 162 of fruiting bodies and organic matter content or pH of the soils could be observed for fungi in a
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8 163 several studies (Falandysz and Bielawski, 2007; Falandysz and Brzostowski, 2007). No such
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10 164 relation could be observed also between soils organic matter content or pH and metals such as Cd
11
12 165 and Pb sequestered in fruiting bodies of several fungi (Gast et al., 1998), as well as of Ag, Al, Ba,
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14 166 Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Sr, Pb, Rb and Zn by Pison Pax, *Paxillus*
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16 167 *involutus*, mushroom (Brzostowski et al., 2011a), while uptake and sequestration in fruiting
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18 168 bodies of metallic elements essential to fungi (K, Na, Ca, Mg, Zn, Cu) is largely regulated by
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20 169 species. In other words, either organic matter content or pH of soils was found as not important
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22 170 factors that can determine the trace metals bioavailability to fungi. Nevertheless, in the complex
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24 171 process of minerals gaining and absorption by mycelium, and in case of mycorrhizal fungi theirs
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26 172 further transfer to symbiotic plant (tree) and also transfer and sequestering in fruiting bodies there
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28 173 are an unknown underlying variables.

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34 174 The soils Hg concentrations increase moving from the north to the south of Poland
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36 175 (Figure 3). This spatial trend can be related to the degree of industrialization and urbanization of
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38 176 the areas surveyed. Dolnośląskie Voivodeship (the sites nos. 11–14; Fig, 1) is a highly
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40 177 industrialized and urbanized region in Southern Poland and differences in soils bedrock
41
42 178 composition between the regions. Furthermore, industrial activities are predominant in
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44 179 Świętokrzyskie Voivodeship (the sites nos. 15 and 16; Southern Poland), where soil Hg
45
46 180 concentrations varied considerably (Figs. 1 and 3, Table 1). Of the 18 locations surveyed in this
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48 181 study, 17 locations exhibited no clear relationship between Hg concentration in Bay Bolete and
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50 182 corresponding soil Hg content. At Karpacz (13 K), a tendency of increasing content of Hg in
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52 183 caps/stipes with increasing soil Hg content was found.

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3 184 A potential of minerals uptake by fungus from the soil and further sequestration in
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5 185 fruiting bodies is assessed using the common concept of bioconcentration factor (BCF) or
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7
8 186 otherwise transfer factor or enrichment factor. They are simply the mushroom's constituent
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10 187 concentration divided by the soil (or other substratum media) mineral constituent concentration.
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12 188 The value of BCF for a given trace element is a measure of its site-specific bioavailability for
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14 189 fungus.

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17 190 Elevated concentration of Hg in topsoil and in parallel a good bioavailability are the
18
19 191 reasons for great concentrations of Hg in fruiting bodies of Bay Bolete at the Złotoryja site (Table
20
21 192 1; site 12 Z). However, for the two highly contaminated specimens from the Włoszczowa site (15
22
23 193 W; containing 2.7 and 1.4 $\mu\text{g Hg g}^{-1}$ dw in caps and 0.89 and 0.87 $\mu\text{g Hg g}^{-1}$ dw in stipes), the
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25 194 driving force for elevated concentrations seems to be great Hg bioavailability (BCF was 80 and
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27 195 16 for caps and 26 and 10 for stipes).

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29 196 Hg content of topsoil from the Karpacz site was elevated (compared to the other locations
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31 197 surveyed), although Hg concentration in Bay Bolete in that site was low. This can be attributed to
32
33 198 low bioavailability of Hg at this site. Content of methylmercury (MeHg) and its proportion to
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35 199 inorganic Hg in soils can impact amount of total Hg sequestered in fruiting bodies (Fischer et al.,
36
37 200 1995). At the Włoszczowa site (15 W), relatively elevated Hg content was found (0.28 and 0.17
38
39 201 $\mu\text{g g}^{-1}$ dw) in topsoil, while concentrations in caps (0.081 and 0.073 $\mu\text{g g}^{-1}$ dw) and in stipes
40
41 202 (0.017 and 0.040 $\mu\text{g g}^{-1}$ dw), were low (BCF between 0.3 and 0.4 for caps and 0.06 and 0.2 for
42
43 203 stipes). Thus, the concentrations of Hg accumulated in fruiting bodies varied widely ($p < 0.05$;
44
45 204 Mann-Whitney U test) among the locations surveyed (Table 1).

52 205 ***Bioconcentration potential***

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55 206 Our results indicate that Bay Bolete can bioconcentrate Hg from the soil. Mercury was found in
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57 207 Bay Bolete, even in unpolluted areas where the BCF (bioconcentration factor) in caps was up to
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3 208 11±6 (Table 1). Bioconcentration factor or transfer factor (TF) is a quotient of Hg concentration
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5 209 in caps or stipes to soil Hg concentration. BCF value below 1 suggests exclusion or bio-exclusion
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8 210 whereas the value above 1 suggests bioconcentration. The values of BCF of Hg in Bay Bolete
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10 211 decreased with increasing soil Hg concentration (Figure 4).

12 212 *Hg intake rates*

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15 213 Both cap and stipe of Bay Bolete are edible and accumulation of Hg in both parts of this
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17 214 mushroom is a human health concern. In a well-grown fruiting body of this fungus, cap is much
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20 215 larger in mass than stipe, while in young specimens, the proportion is approximately equal, and in
21
22 216 much younger specimens, stipe is larger than cap. Nevertheless, there is no published data on Hg
23
24 217 content in caps, stipes or whole fruiting bodies of Bay Bolete collected at different growth stages.
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27 218 The mushroom collectors harvest forest mushrooms daily and individuals, who are late during a
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29 219 mushroom collection day, collect even young fruiting bodies. There are no limits regarding
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31 220 allowable size or allowable daily harvest for mushroom collection or a mushroom species that
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34 221 could be collected by an individual in Poland. For the assessment of daily intake rate of Hg from
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36 222 Bay Bolete, we assumed that the fruiting bodies were collected from mature specimens.

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39 223 Although mushrooms do not constitute a significant portion of human diet, they are
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41 224 popular food items worldwide especially during their seasonal abundance. The data regarding
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43 225 intake rates of wild-grown edible mushrooms at local, regional, national, or international scale
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45 226 are highly limited. For example, as discussed by Zhang et al. (2010), consumption rate of wild-
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47 227 grown mushrooms in Sweden was 1 kg *per capita* annually and Common Chanterelle
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49 228 (*Cantharellus cibarius*) is the most commonly consumed mushroom in that country. In rural
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51 229 areas of Sweden, as well as in rural areas of many other European countries, wild-mushroom
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55 230 picking is a common practice and the intake rates *per capita* are expected to be greater than 1 kg
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58 231 annually. In Czech Republic, 72 % of families take part in wild-mushroom picking, with an
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3 232 average yield of 7 kg fresh weight *per familia* annually, while annual intake rate by some
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5 233 individuals can be up to 10 kg *per capita* fresh weight. In Sichuan Province in China, the annual
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7 234 wild mushroom consumption rate exceeded 20-24 kg *per capita* (Zhang et al., 2010).
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10 235 In Poland, a meal of up to 300 g of fresh mushroom is considered as the common intake
11
12 236 rate in a single dish *per capita* during a week period among mushroom dish fanciers or
13
14 237 individuals with low income rural household. A single dish of up to 500 g of fruiting bodies
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16 238 (fresh weight) collected in wild has been used as daily intake rate of mushrooms *per capita*.
17
18 239 (Zhang et al., 2010).
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21
22 240 Mushrooms, depending on the species, are cooked in several ways. Any mushroom, wild
23
24 241 or cultivated, should not be eaten fresh. Blanching (boiling in water for 10 minutes) of fresh
25
26 242 fruiting bodies is a common procedure. Blanched mushrooms could be further baked, used as
27
28 243 soup ingredients, pickled, or frozen. Dried mushrooms could be grounded (powdered) and used
29
30 244 as a condiment. Dried mushrooms can be soaked in water and added (together with or without
31
32 245 broth) to certain traditional dishes and further cooked without loss of mineral constituents.
33
34 246 Soaking of fresh fruiting bodies of Bay Bolete in salt (2% of table salt per fresh mushroom
35
36 247 weight) water at 20 °C for 5-15 min resulted in 5-10 % loss of total Hg content, while boiling for
37
38 248 15 min resulted in 15 % loss of this element (Svoboda et al., 2001).
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43 249 It is not known if both organic and inorganic forms of Hg are leached during blanching or
44
45 250 other type of processing. A 15 % loss of total Hg contained in fruiting bodies during blanching of
46
47 251 Bay Bolete is considered to be low (Svoboda et al., 2001). Based on the large variation in total
48
49 252 Hg content in Bay Bolete (Table 1), the 15% loss rate from food processing step can be ignored
50
51 253 in the assessment of Hg intake rates by consumers.
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55 254 For the assessment of potential risks from intake of Hg in the fruiting bodies of Bay
56
57 255 Bolete, a reference dose (RfD; 0.0003 mg kg⁻¹ bm daily) and a Provisional Tolerable Weekly
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3 256 Intake values (PTWI; $0.005 \text{ mg kg}^{-1} \text{ bm}$) have been used (JECFA, 1978, US EPA, 1987). The
4
5 257 PTWI of $5 \text{ } \mu\text{g kg}^{-1}$ body weight for total Hg established by the JECFA in 1978 was revised in
6
7 258 2010 (JECFA, 2010). The JECFA established a PTWI of $0.004 \text{ mg kg}^{-1} \text{ bw}$ for inorganic Hg in
8
9 259 2010, based on the assumption that the predominant form of Hg in foods, other than fish and
10
11 260 shellfish, is inorganic Hg. A meal made with 300-500 g of fresh caps of Bay Bolete collected
12
13 261 from the Złotoryja site can result in Hg intake of $0.00025\text{-}0.0004 \text{ mg kg}^{-1} \text{ bw}$ (assuming a 60 kg
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15 262 body weight), based on the median Hg concentration of $0.058 \text{ } \mu\text{g g}^{-1}$ wet weight. If the intake is
16
17 263 calculated from the arithmetic mean of $0.10 \text{ } \mu\text{g Hg g}^{-1}$ wet weight (assuming 90 % water content
18
19 264 in caps), the intake rate will be $0.00043\text{-}0.0007 \text{ mg kg}^{-1} \text{ bw}$.

20
21
22 265 Wild mushrooms are more frequently and at higher rate eaten in a mushrooming season in
23
24 266 late summer and early fall, while usually much less in other periods of the year. In a view of Hg
25
26 267 content, the consumption of Bay Boletes (caps or a whole mushrooms) at the regions surveyed at
27
28 268 least at a rate up to $2.5 \text{ kg per capita}$ weekly in a mushrooming season is safe and will not result
29
30 269 in exceeding of currently allowable Hg intake doses.

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32
33 270 Bay Bolete collected from the Sierakowice site (an unpolluted area) contained the greatest
34
35 271 Hg concentration (Table 1). Consumption of fresh caps of Bay Bolete collected at this site at a
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37 272 rate up to 7.5 kg should not result in Hg intake values exceeding the PTWI limit for a person
38
39 273 weighing 60 kg , assuming that no Hg from other foods is ingested. Forest soil at the Złotoryja
40
41 274 and Karpacz sites contained elevated concentrations of Hg, and Bay Bolete at the Złotoryja site
42
43 275 contained high levels of Hg (Figure 3; Table 1). Consumption of up to 2.4 kg of fresh caps of Bay
44
45 276 Bolete from Złotoryja should not exceed the PTWI limit, if no Hg from other foods is ingested.

46
47
48 277 Selenium in mushroom can provide protective role against Hg (Jarzyńska and Falandysz,
49
50 278 2011b). It is presumed that co-occurrence of selenium and MeHg or total Hg in stoichiometric
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52 279 ratio could prevent the toxic effects of Hg in foods (Yoneda and Suzuki, 1997; Stijve and
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3 280 Cardinale, 1974; Stijve and Besson, 1976). A review of published data on selenium content in a
4
5 281 limited number of Bay Bolete samples collected in Europe indicated that selenium content in
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7
8 282 fruiting bodies usually varies around $0.20 \mu\text{g g}^{-1}$ dw (Falandysz, 2008), which is comparable to
9
10 283 Hg content found in Bay Bolete in this study (Table 1).

11
12 284 The PTWI for MeHg is $0.0016 \text{ mg kg}^{-1}$ bw (JECFA, 2007). We did not analyze MeHg
13
14 285 content in Bay Bolete in this study. Fischer et al. (1995) reported that 0.70 to 1.1 % of total Hg
15
16 286 content in Bay Bolete is in the form of MeHg in a highly contaminated area ($15\text{--}35 \mu\text{g Hg g}^{-1}$
17
18 287 dw). Pilz et al. (2011) in a recent study reported on MeHg content of five species of fungi: an
19
20 288 unknown fungus, *Cordoncella* mushroom (King Bolete?), *Pleurotus djamer*, *P. citrinopileatus*
21
22 289 and *P. ostreatus*, where this compound varied a somehow between the species but largely varied
23
24 290 depending on the extraction method used, i.e. by ultrasonic (from 3 to 38 % of total Hg) or
25
26 291 microwave assisted extraction (from 15 to 41 % of total Hg). Data available on MeHg in wild
27
28 292 mushrooms are sparse and controversial. As reviewed recently, reported MeHg content of King
29
30 293 Bolete (*Boletus edulis*) varied highly (from 0.6 to 39 % of total Hg) between the authors
31
32 294 (Falandysz, 2010).

33 34 35 36 37 38 295 **Conclusions**

39
40 296 Hg concentrations in pooled caps of Bay Bolete collected from unpolluted areas in Poland were
41
42 297 below $1.0 \mu\text{g Hg g}^{-1}$ dw. Mercury concentrations in caps and stipes of Bay Bolete were strongly
43
44 298 correlated ($p < 0.0001$). The relationship between the concentrations of Hg in soil and in
45
46 299 mushrooms varied depending on the location. The bioconcentration potential of Hg in Bay Bolete
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48 300 at sites with elevated soil Hg concentrations was lower than that in soils with low Hg content. In
49
50 301 a view of Hg content, the consumption of Bay Boletes (caps or a whole mushrooms) at the
51
52 302 regions surveyed at least at a rate up to $2.5 \text{ kg per capita}$ weekly in a mushrooming season is safe
53
54 303 and will not result in exceeding of currently allowable Hg intake doses.
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32 **Figure Captions**

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34 501 Figure 1. Locations of the sampling sites of Bay Bolete in Poland. Abbreviations: The communes
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36 502 of Sierakowice (1) and Lipusz in Pomorskie Voivodeship (2); Augustowska Forest in Podlaskie
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38 503 Voivodeship (3); Olsztynek (4) Szczytno in Warmia and Mazury Voivodeship (5); Commune of
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40 504 Olszewo-Borki in Mazowieckie Voivodeship (6); Notecka Forest (7), Porążyn (8), Rogalin (9)
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42 505 Turek in Wielkopolska Voivodeship (10); Lower Silesia Forest (11), Złotoryja (12), Karpacz (13)
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44 506 and Kłodzka Dale in Dolnośląskie Voivodeship (14); Włoszczowa (15) and Starachowickie
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46 507 Forest in Świętokrzyskie Voivodeship (16); Poniatowa in Lubelskie Voivodeship (17);
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48 508 Chochołowska Valley in Małopolska Voivodeship (18).
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55 510 Figure 2. Plot of Hg concentrations in caps against its concentrations in stipes of 221 fruit bodies
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57 511 of Bay Bolete collected from Poland ($y = 0.0231 + 1.4949 * x$; $r = 0.95$; $r^2 = 0.89$; $p < 0.0001$).
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6 513 Figure 3. Median values of Hg concentration in caps or in whole specimens of Bay Bolete and in
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8 514 soil substrate collected under mushrooms: the sampling sites are displayed starting from the north
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10 515 to the south of Poland. Abbreviations: **CS** (1) Mojusz in Commune of Sierakowice and **CL** (2)
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12 516 Commune of Lipusz in Pomorskie Voivodeship; **AF** (3) Augustowska Forest in Podlaskie
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14 517 Voivodeship; **O** (4) Olsztynek and **S** (5) Szczytno in Warmia and Mazury Voivodeship; **O-B** (6)
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16 518 Commune of Olszewo-Borki in Mazowieckie Voivodeship; **NF** (7) Notecka Forest, **P** (8)
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18 519 Porążyn, **R** (9) Rogalin and **T** (10) Turek in Wielkopolska Voivodeship; **LSF** (11) Lower Silesia
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20 520 Forest, **Z** (12) Złotoryja, **K** (13) Karpacz and **KD** (14) Kłodzka Dale in Dolnośląskie
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22 521 Voivodeship; **W** (15) Włoszczowa and **SF** (16) Lipie in Starachowickie Forest in Świętokrzyskie
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24 522 Voivodeship; **P-LV** (17) Poniatowa in Lubelskie Voivodeship; **CH** (18) Chochołowska Valley in
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26 523 Małopolska Voivodeship.
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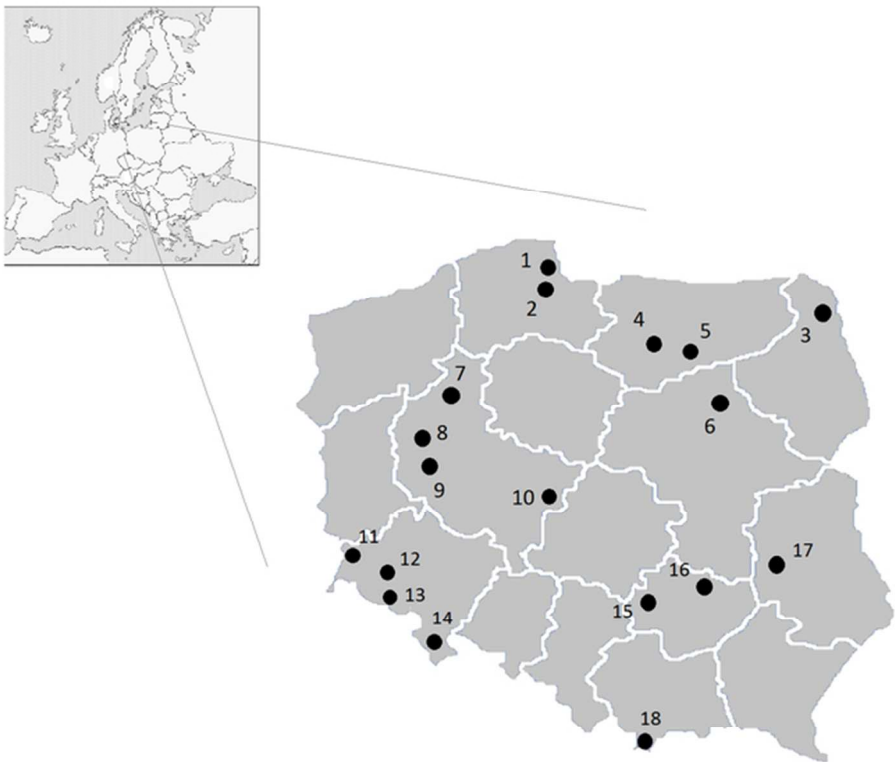
1 Table 1. Mean, standard deviation, range and median values of total mercury concentration in fruiting bodies of mushroom and soil,
 2 and cap to stipe concentration quotient and bioconcentration factor (BCF) values for mercury.

Site, year and number of samples	Hg ($\mu\text{g g}^{-1}$ dw)			Q _{C/S}	BCF	
	Cap	Stipe	Soil		Cap	Stipe
1 (CS) Mojusz, Commune of Sierakowice, Pomorskie Voivodeship, 2007 <i>n</i> = 12	0.32±0.17 (0.13-0.65) 0.29	0.23±0.12 (0.081-0.43) 0.21	0.11±0.09 (0.024-0.26) 0.053	1.4±0.3 (0.95-2.1) 1.4	5.4±5.4 (0.86-17) 3.3	4.1±4.1 (0.42-13) 2.4
2 (CL) Commune Lipusz, Pomorskie Voivodeship, 2006 <i>n</i> = 15	0.14±0.09 (0.080-0.41) 0.10	0.070±0.02 (0.039-0.13) 0.063	0.046±0.033 (0.022-0.14) 0.037	2.0±1.3 (1.3-6.6) 1.6	3.9±2.5 (0.60-8.4) 3.1	2.0±1.3 (0.45-5.1) 1.6
3 (AF) Augustowska Forest, Podlaskie Voivodeship, 2007 <i>n</i> = 15	0.099±0.033 (0.062-0.20) 0.091	0.067±0.020 (0.042-0.12) 0.060	0.075±0.046 (0.020-0.19) 0.046	1.5±0.1 (1.3-1.7) 1.5	2.0±1.4 (0.54-4.5) 1.5	1.3±0.8 (0.37-2.8) 1.2
4 (O) Olsztynek, Warmia and Mazury Voivodeship, 2002 <i>n</i> = 15	0.080±0.020 (0.052-0.13) 0.077	0.044±0.013 (0.031-0.083) 0.040	0.051±0.043 (0.013-0.20) 0.041	1.9±0.4 (1.1-2.5) 1.9	2.2±1.3 (0.29-5.1) 2.1	1.2±0.7 (0.21-2.9) 1.1
5 (S) Szczytno, Warmia and Mazury Voivodeship, 2002 <i>n</i> = 15	0.061±0.013 (0.041-0.093) 0.062	0.040±0.009 (0.029-0.058) 0.039	0.040±0.010 (0.022-0.054) 0.042	1.6±0.2 (1.3-2.0) 1.6	1.6±0.6 (1.0-2.8) 1.4	1.1±0.4 (0.65-2.0) 0.97
6 (O-B) Commune of Olszewo – Borki, Mazowieckie Voivodeship, 2007 <i>n</i> = 15	0.20±0.09 (0.047-0.39) 0.18	0.12±0.06 (0.025-0.22) 0.12	0.026±0.022 (0.0063-0.079) 0.022	1.7±0.6 (1.2-3.8) 1.6	11±6 (1.6-22) 13	7.2±5.2 (1.0-19) 4.4
7 (NF) Notecka Forest, Wielkopolska Voivodeship, 2008 <i>n</i> = 15	0.057±0.028 (0.028-0.13) 0.055	0.048±0.023 (0.007-0.091) 0.043	0.038±0.027 (0.009-0.072) 0.028	1.5±1.0 (0.68-4.4) 0.98	2.0±1.3 (0.46-5.2) 1.7	1.9±1.5 (0.18-5.5) 1.9
8 (P) Porążyn, Wielkopolska Voivodeship, 2008 (15)	0.052±0.025 (0.011-0.092) 0.057	0.031±0.017 (0.009-0.073) 0.032	0.073±0.030 (0.010-0.12) 0.077	1.7±0.5 (0.31-2.8) 1.8	1.1±1.3 (0.16-5.4) 0.78	0.64±0.71 (0.074-3.0) 0.45
9 (R) Rogalin, Wielkopolska Voivodeship,	0.092±0.050 (0.019-0.19)	0.042±0.021 (0.013-0.088)	0.10±0.08 (0.010-0.27)	2.2±0.7 (0.67-3.2)	2.3±2.8 (0.12-8.8)	1.0±1.3 (0.091-4.1)

2008 <i>n</i> = 14	0.088	0.041	0.098	2.3	1.2	0.50
10(T) Turek, Wielkopolska Voivodeship, 2001-2002 <i>n</i> = 11	0.051±0.030 (0.016-0.11)	0.027±0.018 (0.0055-0.057)	0.048±0.006 (0.038-0.057)	2.3±1.4 (1.1-5.0)	1.1±0.6 (0.40-2.0)	0.58±0.38 (0.12-1.1)
11 (LSF) Lower Silesia Forest, Dolnośląskie Voivodeship, 2008 <i>n</i> = 11	0.041	0.027	0.046	1.7	0.76	0.47
11 (LSF) Lower Silesia Forest, Dolnośląskie Voivodeship, 2008 <i>n</i> = 11	0.21±0.21 (0.071-0.78)	0.095±0.095 (0.026-0.37)	0.15±0.10 (0.044-0.41)	2.3±0.9 (1.3-4.2)	1.7±1.7 (0.44-5.0)	0.72±0.62 (0.22-2.3)
12 (Z) Złotoryja, Dolnośląskie Voivodeship, 2008 <i>n</i> = 9	1.0±1.1 (0.38-3.7)	0.68±0.86 (0.21-2.8)	0.37±0.16 (0.18-0.71)	1.0±0.7 (0.13-2.3)	3.2±3.1 (0.82-10)	2.0±2.4 (0.43-7.8)
13 (K) Karpacz, Dolnośląskie Voivodeship, 2008 <i>n</i> = 15	0.58	0.30	0.36	1.0	1.5	1.0
13 (K) Karpacz, Dolnośląskie Voivodeship, 2008 <i>n</i> = 15	0.25±0.14 (0.087-0.53)	0.13±0.06 (0.059-0.24)	0.50±0.20 (0.16-0.77)	2.1±0.9 (0.39-4.5)	0.55±0.31 (0.19-1.2)	0.28±0.13 (0.12-0.49)
14 (KD) Kłodzka Dale, Sudety Mountains, Dolnośląskie Voivodeship, 2001 <i>n</i> = 5**	0.19	0.092	0.46	2.0	0.49	0.28
14 (KD) Kłodzka Dale, Sudety Mountains, Dolnośląskie Voivodeship, 2001 <i>n</i> = 5**	0.26±0.06 (0.18-0.32)		0.15±0.01*** (0.13-0.17)			1.3±0.5 (1.3-2.4)
15 (W) Włoszowa, Świętokrzyskie Voivodeship, 2007 <i>n</i> = 15	0.26		0.14	ND		1.8
15 (W) Włoszowa, Świętokrzyskie Voivodeship, 2007 <i>n</i> = 15	0.36±0.73 (0.064-2.7)	0.17±0.31 (0.017-0.88)	0.068±0.073 (0.011-0.28)	2.7±1.0 (1.6-4.8)	9.3±20.0 (0.29-80)	3.8±7.2 (0.059-26)
16 (SF) Lipie, Starachowickie Forest, Świętokrzyskie Voivodeship, 2000 <i>n</i> = 15	0.098	0.042	0.047	2.5	2.3	1.1
16 (SF) Lipie, Starachowickie Forest, Świętokrzyskie Voivodeship, 2000 <i>n</i> = 15	0.11±0.03 (0.073-0.20)	0.084±0.040 (0.038-0.17)	0.14±0.05 (0.074-0.21)	1.5±0.4 (0.55-2.1)	0.87±0.39 (0.34-1.7)	0.68±0.49 (0.18-2.2)
17 (P-LV) Poniatowa, Lubelskie Voivodeship, 2008 <i>n</i> = 15	0.11	0.075	0.15	1.6	0.75	0.51
17 (P-LV) Poniatowa, Lubelskie Voivodeship, 2008 <i>n</i> = 15	0.16±0.04 (0.12-0.2)	0.082±0.028 (0.062-0.13)		2.0±0.5 (1.7-2.1)		
18 (CH) Chochołowska Dale, Małopolska Voivodeship, Tatra Mountains, 2000 <i>n</i> = 9	0.14	0.070	ND	1.7	ND	ND
18 (CH) Chochołowska Dale, Małopolska Voivodeship, Tatra Mountains, 2000 <i>n</i> = 9	0.24±0.10 (0.10-0.42)	0.15±0.01 (0.13-0.17)		1.5±0.4 (0.84-2.4)		
	0.24	0.14	ND	1.5	ND	ND

Notes: * Number of samples and number of specimens (in parentheses); ND, not determined; ** a whole fruiting bodies; *** 15 samples

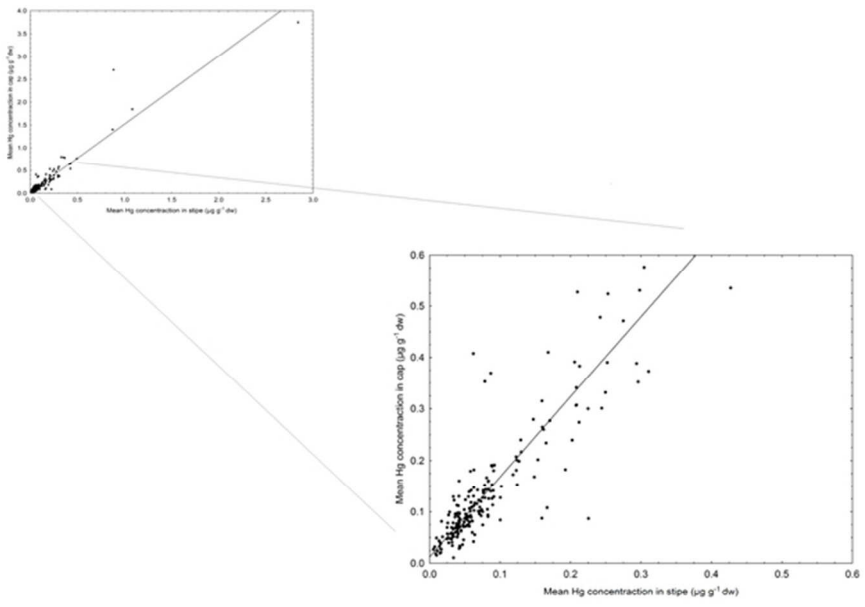
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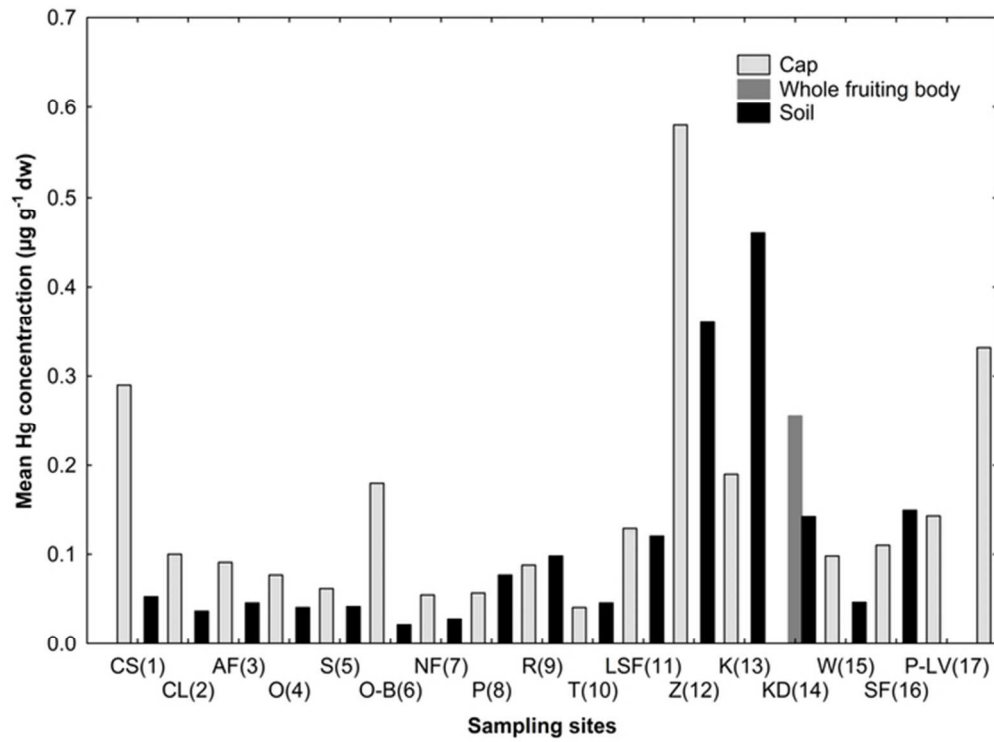
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