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Multi-element survey of wild edible fungi and blackberries in the UK

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Multi-element survey of wild edible fungi and blackberries in the UK

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For Peer Review Only

Abstract

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3 *A survey of 12 metals including lead (Pb), copper (Cu), cadmium (Cd), mercury (Hg),*
4 *arsenic (As) and platinum(Pt) was carried out using ICP-MS in 34 samples of wild*
5 *fungi and 48 samples of wild blackberries collected from sites across the UK. On a*
6 *fresh weight basis (mg/kg) levels of Pb were in the range 0.003-5.990, Cu 0.596-*
7 *34.800, Cd <0.001-19.6, Hg <0.001-4.150, As 0.001 – 0.972 and Pt (µg/kg) 0.006 –*
8 *0.200, with higher concentrations found in fungi than in blackberries.*
9 *The results of the survey showed that the concentrations of the metals were consistent*
10 *with previous studies, where available. Concentrations in wild fungi of Pt, tin (Sn),*
11 *and titanium(Ti) were significantly higher at urban sites than at rural sites. Urban*
12 *blackberries had significantly higher levels of Pb, Ti, and Cd than rural ones, but*
13 *lower levels of manganese (Mn). Pb, Ti and Sn concentrations were significantly*
14 *higher in blackberries sampled near main roads rather than in rural areas.*

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1 **Introduction**

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3 The survey aimed to provide an index of levels of environmental pollution, and to
4 provide a baseline against which to monitor future changes in pollution. Levels of
5 arsenic, cadmium, chromium, copper, lead, manganese, mercury, nickel, platinum,
6 tin, titanium and zinc were measured. It also assessed the levels in the most popular
7 types of wild food collected and eaten in the UK, but not seafoods or game, which are
8 now often farmed and are included in other MAFF surveys (Veterinary Residues
9 Committee, 2002). Therefore, the chosen wild foods were fungi and blackberries.
10 Edible wild fungi were selected because they can accumulate contaminants from the
11 soil (e.g. cadmium, mercury and lead). The accumulation of contaminants by fungi is
12 dependant both on environmental factors such as metal concentrations in the soil and
13 pH, and on factors such as fungal structure (Garcia *et al.* 1998). Several studies have
14 shown that the fruiting bodies (i.e. mushrooms) of many fungal species can
15 accumulate mercury, cadmium and lead (Kalac *et al.* 1991, Garcia *et al.* 1998, Kalac
16 *et al.* 1996, Falandysz *et al.* 2004), with some very high lead concentrations being
17 reported in mushrooms growing in the vicinity of highways or other sources of lead
18 (Kalac *et al.* 1996).

19
20 Wild blackberries were analysed as indicators of surface pollution (e.g. from traffic
21 exhausts). There have been recent concerns about the potential for increases in the
22 levels of platinum in the environment due to emissions from catalytic converters in
23 car exhausts (Anon, 1998, Farago *et al.*, 1998, Hees *et al.*, 1998, Ravindra et al,
24 2004). At the time of sampling, it is believed that approximately half the cars
25 registered in the UK had catalytic converters fitted, whereas virtually none was fitted
26 before 1993. Platinum was therefore included in this survey to compare its levels of
27 contamination in wild foods growing close to roads with those grown away from
28 traffic.

29
30 Manganese was also included in this survey in order to provide baseline data against
31 which any increase in contamination can be compared, should manganese-based fuel
32 additives be used in the future as replacements for leaded fuel. Titanium was
33 analysed as an indicator of soil contamination. Plants do not take up titanium

1 internally, and so the presence of titanium in plant samples can be used to indicate the
2 presence of soil or dust on the outer surface of the plant sample.

3
4 Samples of wild fungi and blackberries were taken from both rural and urban
5 locations, and from roadside sites and those distant from roads around the UK in order
6 to reflect potential localised sources of contamination. There are no data for wild
7 foods from previous UK surveys, however several surveys have measured elements in
8 commercially-grown mushrooms and blackberries (MAFF, 1982, 1983, 1985,
9 1987a,b, 1997a). Such commercial samples are likely to have been produced under
10 much more controlled conditions and, for fungi, comprise different species to some of
11 the wild fungi in this survey.

12
13 Some metals and other elements (e.g. zinc, selenium, cobalt, copper) can act as
14 nutrients and are essential for health, while others (e.g. mercury, cadmium, lead) have
15 no known beneficial health effects (MAFF, 1998 a,b). All may be harmful if
16 excessive amounts are consumed. Metals and other elements are present in foods
17 naturally; as a result of human activities (e.g. agricultural practices, industrial
18 emissions, car exhausts); from contamination during manufacture/processing and
19 storage; or may be added directly (MAFF, 1998 a,b, Peshin et al., 2002).

20 21 22 **Materials and methods**

23
24 The survey was undertaken between June and November 1998. Samples (ideally at
25 least 200 g) of wild fungi and blackberries were collected from the 'North', the
26 'Midlands' and the 'South' of mainland Great Britain.

27 28 *Fungi samples*

29
30
31 Samples were collected by Members of the British Mycological Society, with half
32 from urban and half from rural locations. These volunteers were used
33 as types of edible fungi can be difficult to find and to identify. They were provided
34 with written instructions on sample collection. Records included the date of

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1 collection, the ordnance survey grid reference and whether the sample was from a
2 'rural' or 'urban' location. The volunteers were asked to obtain mushrooms, mainly
3 of '*Agaricus*' species, which are the most commonly eaten wild fungi. Where this was
4 not possible, other common edible species, which they would also normally collect,
5 could be substituted. The Latin and common names of the fungi collected are given
6 in table 1. Once collected, the fresh samples were sealed in air and water tight plastic
7 bags. More samples of wild fungi than the planned 24 were collected, and so all 34
8 samples were analysed in order to give a better representation of results. A minimum
9 of 25g of fresh mushroom was required for analysis but collectors were requested to
10 provide at least 50g.

11
12 [table 1 about here]

13 14 *Blackberry samples*

15
16 The survey was designed to collect 48 samples of wild blackberries. Within the
17 three regions, four samples each came from 'rural' sites, 'urban' sites, 'beside a rural
18 road' and 'beside a main road'. Once collected, the samples were washed with
19 distilled water, dried using absorbent paper (Kimwipes Classic (Steel Blue),
20 Kimberly-Clark) and then sealed in plastic bags prior to analysis. A minimum of 200g
21 fresh weight were collected for each sample.

22 23 *Soil samples*

24
25 A sample of the soil substrate was collected from each site of fungi and blackberry
26 collection and this comprised random sub-samples taken from within the area of the
27 site to a depth of 15 cm, using a soil auger. The soil samples were stored in sealed
28 plastic sample bags. The soil samples were not analysed, but were stored against the
29 need for analysis to investigate any unexpected or unusual results.

30 31 *Analysis*

32

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3 1 The wild fungi and blackberry samples were prepared and analysed for arsenic,
4 2 cadmium, chromium, copper, lead, manganese, mercury, nickel, platinum, tin,
5 3 titanium, and zinc.
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9 5 All samples were washed thoroughly with distilled water and the edible portions
10 6 prepared. Aliquots of the homogenised edible portion of each sample (equivalent to
11 7 approximately 0.5 g dry weight) were digested with nitric acid by microwave heating
12 8 in a CEM MDS 2000 UDV system. All elements were measured by inductively
13 9 coupled plasma-mass spectrometry (ICP-MS) using a Perkin Elmer Elan 6000. All
14 10 measurements were covered by the United Kingdom Accreditation Service (UKAS)
15 11 accreditation, except titanium which was used as a screen only and thus outside the
16 12 scope of UKAS accreditation, and platinum, which was analysed by a different
17 13 method in order to obtain a lower limit of detection (LOD). For the platinum
18 14 samples, aliquots of each sample (equivalent to approximately 0.5 g dry weight) were
19 15 digested a form of aqua regia (4+1, concentrated nitric and hydrochloric acids (v/v))
20 16 by microwave heating in a Perkin Elmer Multiwave microwave digestion system.
21 17 This method was chosen because platinum that is emitted from car exhausts fitted
22 18 with catalytic converters is likely to be in resilient forms that are difficult to digest
23 19 fully by nitric acid digestion alone. However, this method was outside the scope of
24 20 UKAS accreditation.
25
26 21

22 *Quality Control*

23

24 The approach for quality control in multi-element analyses by the analyst has been
25 25 discussed elsewhere (Baxter *et al.* 1997). Every multi-element analysis in this study
26 26 included the following quality control checks:
27

- 28 • Measurement of a calibration standard at the start and end of each ICP-MS run –
29 values to be within $\pm 20\%$ of each other;
- 30 • Spiked reagent blank recoveries to be within $\pm 20\%$ of the expected value (and all
31 results are corrected for spike recovery);
- 32 • Replicate determinations to give a relative standard deviation of $\leq 20\%$ or twice
33 the LOD; and

- 1
2
3 1 • Certified Reference Material (CRM) results – values to be within the certified
4 2 range or $\pm 40\%$ of the certified value, whichever was the greater.
5 3

6
7 4 Every microwave digestion batch included a National Institute of Standards
8 5 Technology (NIST) certified reference material (CRM) (BCR 60 – Aquatic Plant;
9 6 BCR 61 – Aquatic Moss; NIST 1547 – Peach leaves; NIST 1570 – Spinach; NIST
10 7 1573 – Tomato leaves) and a duplicate sample. Each batch also contained reagent
11 8 blanks, and a reagent blank spiked with a known amount of each element for recovery
12 9 estimate purposes. Rhodium and indium were added to the digests as internal
13 10 standards before element concentrations were measured by ICP-MS.
14 11

15 12 The limits of detection of the elements analysed in the fungi and blackberries
16 13 surveyed are shown in table 2.
17 14

18 15 [table 2 about here]
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21 18

22 19 **Results and discussion**

23 20 There were detectable levels of titanium on all the samples, indicating contamination
24 21 by soil. For blackberries, this was significantly greater for samples obtained next to
25 22 major roads, presumably as a result of splashing from vehicle wheels in wet weather.
26 23 Titanium concentrations in urban fungi samples (1.4 mg/kg) were found to be
27 24 significantly higher ($P < 0.05$) than in rural samples (0.35 mg/kg), however, it should
28 25 be noted that species of fungi were not matched between the two types of site. For
29 26 blackberries, the highest concentration of 1.8 mg/kg was found in an urban roadside
30 27 sample and, as with the fungi, titanium was significantly higher ($p < 0.01$) in the urban
31 28 samples.
32 29

33 30 The results for titanium suggest that, not surprisingly, significant levels of soil and
34 31 dust may remain in the wild fungi and berry samples even after washing with clean
35 32 water. Any surface soil remaining in samples would have contributed to the titanium
36 33 concentrations reported. However, since none of the reported concentrations were
37 34 above guideline limits, this was not pursued.
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5 2 The results for each element are shown in tables 3 for wild fungi and 4 for wild
6 3 blackberries. Further details can be found elsewhere (University of Bristol, 1999;
7 4 MAFF 2000) . All results are reported on a fresh weight basis.
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9 6

10 [tables 3 and 4 about here]
11 7
12 8

13 8 Several of the samples for both fungi and blackberries contained relatively high
14 9 concentrations of some elements, and highly skewed distributions of values were
15 10 obtained. This is consistent with previous studies (Kalac *et al.*, 1991, Kalac *et al.*,
16 11 1996, Barcan *et al.*, 1998, Garcia *et al.*, 1998, Michelot *et al.*, 1999).
17 12
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19 *Fungi* 20 14 21 15

22 15 Over half the samples collected were *Agaricus* species. No urban samples were
23 16 collected from the north but two extra samples were collected from each of urban
24 17 midlands and urban south. These four extra samples were used in an analysis of urban
25 18 – rural differences in order to maintain the urban/rural balance within the survey.
26 19

27 19 Several samples of wild fungi did show the ability to accumulate some relatively high
28 20 concentrations of certain elements, especially cadmium, copper, arsenic, mercury,
29 21 zinc and lead. This is consistent with earlier studies, which show that certain fungi
30 22 can accumulate very high concentrations of metals (Anon, 1998, Barcan *et al.*, 1998,
31 23 Farago *et al.*, 1998, Garcia *et al.* 1998, Hees *et al.*, 1998, Kalac *et al.* 1991, Kalac *et*
32 24 *al* 1996, Michelot *et al.*, 1999, Falandysz *et al.* 2004).
33 25
34 26

35 26 Overall, there appeared to be few large or significant differences between samples
36 27 from the rural and urban sites. Statistical analysis showed samples of fungi from
37 28 urban sites contained significantly higher concentrations of titanium, tin and platinum
38 29 and lower levels of zinc (Mann Whitney, exact P values = 0.014, 0.019, 0.003, 0.028
39 30 respectively).
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42 *Blackberries* 43 33 44 34 45 35 46 36 47 37 48 38 49 39 50 40 51 41 52 42 53 43 54 44 55 45 56 46 57 47 58 48 59 49 60 50

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1 In general, the concentrations of the chemical elements in the samples of blackberries
2 were low, with many falling below the limits of detection. Kruskal Wallis tests were
3 used on the non-parametric data to test for regional and site variation. There were few
4 regional differences but levels of arsenic and chromium were significantly lower ($P <$
5 0.001) in the north than in the midlands and south of the UK. Levels of copper were
6 also lower in the north but platinum was higher in the samples from this region ($P <$
7 0.01). Manganese concentrations were significantly higher ($P < 0.001$) in samples
8 from rural locations, with median levels of 11.7 mg/kg almost six times as high as
9 urban samples (Table 4). Manganese is commonly applied to agricultural crops,
10 which are often deficient in this trace element. Mean concentrations of lead were
11 almost nine times higher ($P < 0.001$) in samples by major roads and at urban sites than
12 rural sites. Significantly elevated levels of tin ($P < 0.01$), chromium ($P < 0.05$),
13 arsenic ($P < 0.05$) and titanium ($P = 0.001$) were also found in blackberries in those
14 locations. These results for lead and platinum are as expected because road traffic
15 represents a significant localised source of emissions of these elements to the
16 environment.

17
18 The levels of the four elements (arsenic, cadmium, mercury and lead) with the
19 greatest potential to be toxic to humans are illustrated for edible fungi in figure 1 and
20 for blackberries in figure 2, with further comments on these results given below.

21
22 [Figures 1 and 2 about here]

23 24 *Arsenic*

25
26 Several samples of fungi showed high arsenic levels, the highest being in *Agaricus*
27 *macrosporus* (0.97 mg/kg), *Lepiota procera* (0.83 mg/kg) and *Agaricus arvensis* (0.73
28 mg/kg). These concentrations are, nonetheless, below the statutory limit of 1 mg/kg
29 for arsenic in commercial foods set by *The Arsenic in Food Regulations 1959*, as
30 amended, although those Regulations do not apply to wild foods collected for
31 personal consumption. There was no significant difference between arsenic
32 concentrations in urban and rural samples of fungi.

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1 Levels of arsenic were very low in all the blackberry samples with the highest
2 concentration being 0.069 mg/kg. The results show significantly ($P < 0.05$) higher
3 concentrations in urban samples (mean 0.0102 mg/kg, range 0.0015 mg/kg to 0.0690
4 mg/kg) than rural samples (mean 0.0032 mg/kg, range 0.0011 mg/kg to 0.0079
5 mg/kg). However, there were no significant differences in arsenic levels between
6 roadside and non-roadside samples of blackberries.

7 8 *Cadmium*

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10 Cadmium concentrations in wild fungi ranged from 0.02 mg/kg to 20.0 mg/kg with a
11 mean of 1.6 mg/kg. Relatively high concentrations of cadmium were found in several
12 species (*Agaricus macrosporus*, 20 mg/kg; *Agaricus campestris*, 8.7 mg/kg; and
13 *Agaricus arvensis*, 5.3 mg/kg). This could reflect either a higher rate of cadmium
14 accumulation in these species, or it could be that these samples happened to be
15 growing in soil that had higher levels of cadmium.

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17 Some results from this study are somewhat higher than those from a study of wild
18 fungi undertaken in Croatia (Mandic et al., 1992) (mean 1.2 mg/kg, range 0.3 mg/kg
19 to 4.87 mg/kg dry weight) although different species were analysed. The mean
20 concentration of 1.6 mg/kg from this study is greater than the mean concentration of
21 0.018 mg/kg found in samples of commercial fungi reported elsewhere (MAFF 1997)
22 and also in 4 samples of mushroom in an earlier study of background levels of
23 cadmium in foods (MAFF 1983) where the mean concentration was 0.06 mg/kg (0.02
24 mg/kg to 0.08 mg/kg). Directive 2001/22/EC sets a limit of 0.2 mg/kg wet weight for
25 Cd in all cultivated fungi. Fourteen out of 34 samples exceeded this limit, which is not
26 applicable to wild fungi.

Comment [C1]: Toby – can we give the exact numbers or proportion of samples over 0.2 mg/kg?

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28 Cadmium concentrations in the blackberry samples ranged from 0.0007 mg/kg to
29 0.094 mg/kg with a mean concentration of 0.011 mg/kg. There was no significant
30 difference between the urban and rural samples or between roadside and non-roadside
31 samples. A MAFF study analysing foods grown in the Shipham area (MAFF 1983)
32 where elevated levels of cadmium are found as a result of historic mining activity,
33 found a commercial sample of blackberry with a cadmium level of 0.08 mg/kg, which
34 is at the higher end of the range found in samples from this survey. Although not a

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1 direct comparison, strawberries analysed in a Finnish study (Tahvonen et al., 1995)
2 had a mean cadmium concentration of 0.007 mg/kg, which is similar to concentrations
3 in this survey.

4 5 *Lead*

6
7 Lead was detected in all of the wild fungi samples (mean 0.7 mg/kg, range 0.012
8 mg/kg to 6 mg/kg). The majority of the samples had lead concentrations below the
9 EU statutory limit of 0.3 mg/kg for lead in commercial fungi (Directive 2001/22/EC),
10 although, as noted above for arsenic, these statutory limits do not apply to wild foods
11 collected for personal consumption. Five samples with higher lead concentrations
12 were found, with the greatest (6.0 mg/kg) being in a sample of 'Wrinkled Club'
13 (*Clavaria* species). Statistical analysis showed there to be no significant difference
14 between urban and rural samples.

15
16 The mean concentration from this survey (0.7 mg/kg) is greater than results for
17 commercial fungi tested in an earlier JFSSG study, (MAFF, 1997a) where a mean
18 concentration of 0.01 mg/kg was obtained. However, this result is expected as
19 commercial fungi are different species, grown in controlled conditions and are
20 unlikely to be exposed to the same types of contaminant as those growing in the wild.
21 This has been shown in a previous study (Borella et al., 1994). A study in Croatia
22 measured a range of lead concentrations in edible wild fungi up to a high of 7.72
23 mg/kg (Mandic et al., 1992).

24
25 None of the wild blackberry samples had lead concentrations above 1 mg/kg. There
26 was no significant difference between the samples taken from urban sites and main
27 road sites. However, the mean concentration of lead in urban samples (0.093 mg/kg)
28 was significantly greater ($P < 0.01$) than in the rural samples (0.012 mg/kg), which
29 suggests that localised environmental contamination in urban areas and near main
30 roads does contribute to higher lead levels in wild blackberries. The mean
31 concentration of lead found in blackberries in this survey (0.05 mg/kg) is less than
32 that found in commercial fresh blackberries (0.07 mg/kg) in a previous survey
33 (MAFF, 1987a).

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1 *Mercury*

2
3 The concentrations, on a fresh weight basis, in all of the wild fungi samples were
4 above the LOD of 0.0006 mg/kg (mean 0.64 mg/kg, range 0.006 mg/kg to 4.2 mg/kg)
5 and there was no significant difference in levels of mercury between urban and rural
6 samples of fungi. Mean levels measured were greater than those for commercial
7 fungi (mean 0.02 mg/kg) in a survey of individual foods (MAFF, 1987b). However,
8 as noted above, commercial fungi samples may be different species and are grown in
9 controlled conditions and so this difference is not surprising. Falandysz et al. (2004)
10 found that bioconcentration factors of different species of wild edible mushroom
11 sampled in Poland varied on average between none and 73 times the concentration of
12 the soil substrate. The range for the majority of species was between 4 and 23 in the
13 caps. Actual values (on a dry matter basis) were between 0.17 mg/kg in the stalks and
14 0.92 mg/kg in the caps. They concluded from their study that even at a high
15 consumption level of 23 g per day of the most contaminated species (*Amanita*
16 *muscaria*), there was no risk to human health.

17
18 In wild blackberries, the mercury concentrations were very low (mean 0.00088
19 mg/kg, range 0.0006 mg/kg to 0.0079 mg/kg) with only 8 samples having mercury
20 concentrations above the LOD of 0.0006 mg/kg.

21
22 **Conclusions**

23
24 The levels of potentially harmful elements in wild foods were in general these low,
25 and often below the limit of detection. Concentrations of tin and titanium were
26 significantly higher at urban sites than at rural sites in both wild fungi and
27 blackberries. Platinum concentrations in blackberries were significantly higher at
28 roadside locations. Levels of platinum are believed to reflect its use in catalytic
29 converters and could be used to monitor future pollution. Levels of lead, which were
30 significantly greater in blackberries sampled within urban areas and adjacent to main
31 roads, might be expected to decrease in future, as lead is no longer added to petrol in
32 the UK.

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Table 2. Limits of Detection (LOD)

ELEMENT	SYMBOL	LOD (mg/kg)*
Arsenic	As	0.0005
Cadmium	Cd	0.0001
Chromium	Cr	0.01
Copper	Cu	0.002
Lead	Pb	0.0006
Manganese	Mn	0.01
Mercury	Hg	0.0006
Nickel	Ni	0.002
Platinum	Pt	0.00001**
Tin	Sn	0.001
Titanium	Ti	0.001
Zinc	Zn	0.04

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* fresh weight

NB The LODs shown above are for a nominal sample weight of 4g, as taken in this survey

** For the wild blackberries, a more sensitive procedure using *aqua regia* digestion was used for which an LOD of 0.000006 mg/kg was obtained (see text)

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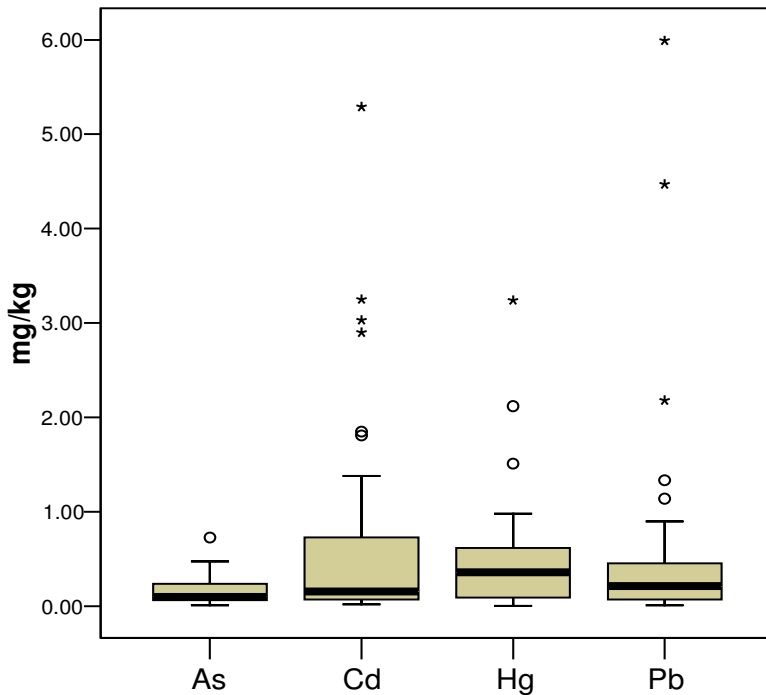
1 Table 3. Summary statistics of the levels of the elements found within the mushroom
2 samples (mg/kg fresh weight).
3

	N	Median	Range	Minimum	Maximum
Ti	34	0.41	6.03	0.03	6.06
Cr	34	0.04	0.96	0.01	0.97
Mn	34	1.44	8.36	0.45	8.81
Ni	34	0.044	0.348	0.009	0.357
Cu	34	6.0	32.1	2.7	34.8
Zn	34	11.4	31.0	3.6	34.6
As	34	0.109	0.961	0.011	0.972
Cd	34	0.17	19.58	0.02	19.60
Sn	34	0.003	0.079	0.001	0.080
Pt	34	0.00002	0.00019	0.00001	0.00020
Hg	34	0.36	4.14	0.01	4.15
Pb	34	0.22	5.98	0.01	5.99

Table 4. Summary statistics of the levels of the elements found within the blackberry samples (mg/kg (*Pt $\mu\text{g}/\text{kg}$) fresh weight).

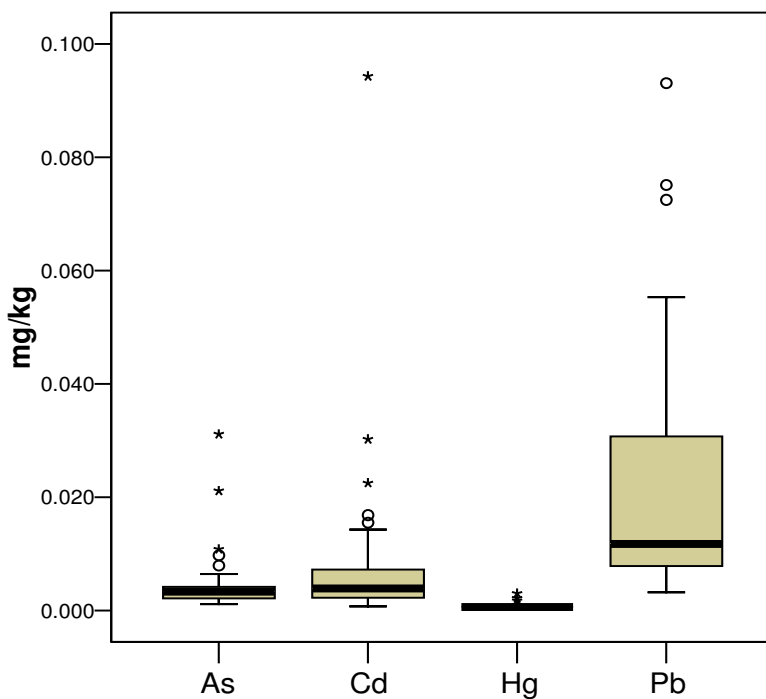
	N	Median	Range	Minimum	Maximum
Ti	48	0.071	1.777	0.033	1.810
Cr	48	0.02	1.23	0.01	1.24
Mn	48	4.61	151.88	1.12	153.00
Ni	48	0.087	0.670	0.025	0.695
Cu	48	0.938	2.694	0.596	3.290
Zn	48	2.55	4.03	1.52	5.55
As	48	0.0035	0.0680	0.0011	0.0691
Cd	48	0.0044	0.0936	0.0007	0.0943
Sn	48	0.001	0.189	0.001	0.190
Pt*	48	0.006	0.070	0.006	0.076
Hg	48	0.0006	0.0073	0.0006	0.0079
Pb	48	0.0128	0.5458	0.0032	0.5490

Figure 1. Levels of arsenic, cadmium, mercury and lead in wild mushrooms. Two extreme sample values for cadmium have not been plotted and were 8.67 and 19.6 mg/kg. Values are fresh weight basis with the centre line of the box representing the median of the distribution, the box the interquartile range. The whiskers extend to 1.5 times the box length with outliers (circles) from 1.5 to 3.0 x box length and extreme values (stars) above 3 x box length.



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Figure 2 Levels of arsenic, cadmium, mercury and lead in wild blackberries. See Fig 1 for explanation of box and whisker plots. Levels of lead (Pb) were variable and five extreme values (0.127, 0.160, .0365, 0.382, 0.549) have not been plotted for clarity. Note the scale is different from fig 1 and values are also on a fresh weight basis.



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