Essential and toxic elements in meat of wild birds

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Running title: Essential and toxic elements in wild birds

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Abstract

Essential and toxic elements were determined by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Mass Spectrometry (MS), and Atomic Absorption (AS) in meat of 14 migratory birds originating from central and northern Europe to provide baseline data regarding game meat consumed in central Italy. In all samples analyzed, cobalt (Co) and chromium (Cr) (total) levels were < 0.326 mg/kg_{ww}. As far as non-essential or toxic elements, arsenic (As), barium (Ba), cadmium (Cd), stannous (Sn), thallium (Tl), tellurium (Te), titanium (Ti), cerium (Ce), lantanium (La), uranium (U) concentrations were < 0.326 $mg/kg_{ww,}$ thorium (Th) < 1.63 mg/kg_{ww} and mercury (Hg)< 0.0163 mg/kg_{ww}. When detectable, lead (Pb) concentrations always exceeded maximal admissible levels for metal (0.1 mg/kg_{ww}) established by the European Commission for meat. These findings indicate that elevated Pb concentrations in game ingested by humans may be a cause for concern.

Keywords: Essential elements, toxic elements, meat, migratory birds

INTRODUCTION

Meat is an important food which is part of a balanced diet contributing valuable nutrients that are beneficial to health. Meat contains high levels of protein, vitamins, minerals and micronutrients which are essential for growth and development (WHO, 1996). The content of nutrients in wild animals are higher than those in farm animals which differ substantially in the type of feed and in habits (Strazdina et al., 2011). In wild animals levels of fats are lower compared to those in farms (Nuernberg et al., 2011). Wild animals travel more extensively than farm bred animals and in particular migratory birds travel prolonged distances requiring a muscle apparatus which is oxygenated, well-developed and contains little fat. Migratory birds display an intensive metabolism and consume large quantities of food per unit of body mass (Torrella et al., 1998).

It is worthwhile noting that meat of wild animals is also an important source of exposure to anthropic contamination including bacterial, chemical and radioactive agents. Chemical contamination of the ecosystem with elements such as arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), antimony (Sb), which are ranked among hazardous substances of high priority (ATSDR, 2005; Desideri et al., 2012; Phillips et al., 2014; Burger and Elbin, 2015), is a serious problem, threatening the habitat and health of wild animals and humans. These elements are natural components of the Earth’s crust and biological systems. Their concentrations were found to be increased in the ecosystem during the last decades due to industrial and other anthropic activities. These activities are a source of aerosol particles and contaminated dust containing “heavy metals” that are brought into the atmosphere and may be transported over long distances, resulting in soil and vegetation contamination of industrialized as well as non-industrialized areas (Wolkers et al., 1994; Dahshan et al., 2013).

It is well established that vegetation is contaminated either by aerial deposition or by absorption of these metals from soil. Contamination of wildlife due to heavy metals occurs by inhalation of small particles but the prevalent route is ingestion of contaminated food. Thus high concentrations of heavy metals were reported in organs of wild birds, even in remote areas (Wolkers et al., 1994). Birds are an important bio-indicator organisms because these species are continuously exposed and may bioaccumulate toxic compounds. Resident birds reflect local level of contamination; while migrating species transport toxicants across long distances. The herbivores of terrestrial fauna, birds as well mammals, demonstrate generally higher levels of toxic elements than carnivores since vegetation is contaminated either by aerial deposition or by absorption of these elements from soil (Pompe-Gotal and Prevendar Crnic, 2002). A main concern consists of transfer of pollutants through food chain pathways which become elevated in game species which is subsequently ingested by humans (Taggart et al., 2011).

A further additional important source of human contamination by food is bullet-derived Pb in game meat (Scheuhammer et al., 1998). Careless removal of tissues from around the bullet pathway in the animal body results in elevated Pb levels from meat ingested by humans (Tagne-Fotso et al., 2016). Because (1) a great number of wild birds are killed each hunting season and this activity yields a significant amount of edible meat, and (2) wild animals are continuously exposed to pollutants, the aim of this study was to examine human and animal health risks related to ingestion of wild bird meat.

The elements taken into account in this study were 31 subdivided into essential, major (Na, K, Ca, Mg, P, S), minor or trace (Mn, Fe, Cu, Zn, Co, Cr and Si) and non-essential or toxic (Al, Ba, Rb, Sr, As, Cd, Sn, Ce, La, Tl, Te, Ti, Th, U, Hg, Sb, Ni and Pb). An element is considered essential if the animal fails to grow normally or to complete its life cycle, if grown in a medium lacking this element. In the presence of the suitable concentration of the element it grows and reproduces normally. Essential metals also produce adverse effects when consumed in high concentrations whereas non-essential metals are toxic even at low levels for humans and environment.

Iron, Cu, Mn and Zn are essential elements participating in enzyme metabolism. These elements exert immunomodulatory functions and thus influence susceptibility and consequences to exposure to a variety of viral infections (Meli et al., 2015). Mn and Zn play a vital role in the control of diabetes (Naga Raju et al., 2006). The importance of Fe in maintaining good health and well-being has long been recognized by nutritionists; meat is the food richest in heme Fe, the iron form with the highest bioavailability (Carpenter and Mahoney, 1992). Meat is indeed the richest source of Zn in the Italian total diet (Lombardi-Boccia et al., 2003) and provides also substantial amounts of Cu. Although Cr (III) is an essential element that enables the body to use sugar, protein and fat, the Cr (VI ionic state) is carcinogenic for organisms (Demirezen and Uruc, 2006).

Arsenic, V, Cd, Pb, Sn, Sb, and Hg have no known physiological function yet reported and are considered as a high risk factor to public health. Cd exerts an adverse effect on brain metabolism and other severe effects on prostate, kidney, liver, lungs and bones (Ginsberg, 2012; Huang et al., 2009). Pb intoxication damages nervous and hemopoietic systems and may also result in dysfunction of renal tubules, liver, and cardiovascular system (Tsuchiva, 1986; Counter et al., 2015; De Almeida et al., 2015). Children are particularly at risk from Pb consumption, both before and after birth (Buchanan et al., 2011; Nacano et al., 2014). Hg is a neurotoxic poison that produces neurobehavioral effects, neuroendocrine and renal damage and gastrointestinal toxicity (Counter et al., 2002; Chen et al., 2011). Arsenic, a problematic element for humans, is well known to exert adverse effects on humans where speciation of As plays an important role in determining toxicity to humans (Desideri et al., 2016; Zavala et al., 2008). Antimony (Sb) and many of its compounds are toxic, and effects of Sb poisoning are similar to

As toxicity (Roselli et al., 2015). The toxicity of Sb is lower than that of As which might be attributed to significant differences in uptake, metabolism and excretion.

Therefore, determination of heavy and transition metals in wild birds is of interest for quality control when considering it as food source. The purpose of this study was to provide information on concentration of essential and non-essential or toxic elements in wild birds

MATERIALS AND METHODS

Samples

Meat (muscle) samples originated from 14 wild birds obtained by Italian hunters in autumn and winter 2012-2014 in different countries of Europe (Central Italy, Croatia, Greece, Hungary, Romania and Sweden). One sample (pheasant-*Phasianus colchicus*) was a not migratory bird, while all remaining were migratory birds. Only the trush was a passerine species, while all the remaining were not passerine. Table 1 shows the samples analyzed, species, the family, hunting site and year.

Sample pretreatment

After removal of pens, skin and bones, meats were grounded. A suitable amount (about 150 g) was weighed, frozen at -20° C and the next day, freeze-dried for 24 hr using a freeze dryer module EDWARDS. The dehydrated samples were weighed and homogenized. The dry weight was used as wet weight values were variable. The mean ratio between dry and wet weight (dw/ww) was 0.33 ± 0.08.

Sample dissolution

The sample dissolution was carried out following the method EPA 3050B 1996 proposed by the Environmental Protection Agency. A half (0.5 g) (dry weight) of sample was digested in a mixture of 3 ml concentrated nitric acid, 10 ml concentrated hydrochloric acid and 1 ml 30% hydrogen peroxide. The sample was refluxed for 1 hr at 95°C in the block digestor DigiPREP

MS (SCP Science, Canada). The digestate was filtered through 0.45 µm pore size filters paper; after washing, the filtrate was collected obtaining a 1% solution. All chemicals used in sample treatment were suprapure grade; ultrapure water was used for all solutions.

Measure methods

Element determination was carried out following three methods (Table 2): a) EPA 6010D 2014 for measurement of K, Na, Ca, Mg, P, S, Mn, Fe, Cu, Zn, Co, Cr (total) Si, Al, Ba, Sr, As, Cd, Sn, Tl, Te, Ti, Th, Sb, Ni and Pb; b) EPA 6020B 2014 for determination of Ce, La, Rb and U and c) EPA 7470A 1994 for measurement of Hg.

In the first method (EPA 6010D 2014), element determination was carried out by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) that may be used to determine trace elements in solution. In this method multi-elemental determinations by ICP-AES used simultaneous optical systems and axial viewing of plasma. The instrument (ICP-720-Es, Varian, California) measures characteristic emission spectra by optical spectrometry. Samples are nebulized and resulting aerosol transported to the plasma torch. Element-specific emission spectra are produced by radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer, and intensities of emission lines monitored by photosensitive devices. The minimum detectable concentration (MDC) was 0.326 mg/kg_{ww} for all elements determined.

In the second method (EPA 6020B 2014) Ce, La, Rb and U determination was carried out by measurement of ions produced by radio-frequency inductively coupled plasma (ICP MS) using an X Series II ICP-MS (Thermo Fisher Scientific Inc, NYSE:TMO) with an Octopole Reaction System. Analyte species originating in a liquid are nebulized and resulting aerosol transported by argon gas into the plasma torch. The ions produced by high temperatures are entrained in the plasma gas and introduced, by means of an interface, into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier. The MDC was 0.652 mg/kg$_{ww}$ for all elements determined.

Hg was determined by EPA 7470A 1994. This technique is based upon the absorption of radiation at 253.7-nm by Hg vapor. The Hg is reduced to the elemental state and aerated from solution in a closed system. The Hg vapor passes through a cell positioned in the light path of an atomic absorption spectrophotometer (SpectrAA220 Fast Sequential, Varian, California). Absorbance (peak height) is measured as a function of Hg concentration. The MDC was 0.0163 mg/kg$_{ww}$.

Quality control

A blank sample was also prepared, in order to take into account possible impurity of reagents and release from containers. Interferences need to be assessed and valid corrections applied or data flagged to indicate problems. The accuracy of the method was evaluated using recovery tests with a Laboratory Control System (LCS) constituted by a blank sample added with known quantities of analytes. The averaged analytical standard errors observed with respect to reported certified materials ranged between 10-15% and 8-12% for EPA 3050B1996+EPA 6010D 2014 and EPA 3050B1996+EPA 6020B 2014 respectively.

RESULTS

Tables 3-5 show, for each meat sample, measured concentrations (mg/kg$_{ww}$) of the element taken into account, arithmetical mean concentration with relevant standard deviation, minimum and maximum values. For mean calculation, values below MDC not were considered. All element concentrations in tissues were measured and referred to as wet weight (ww). To report the concentration in wild bird muscle as wet weight (ww), a conversion factor of 0.33 was used (0.33 = the arithmetical mean of the ratios between dry and wet weight).

As far as non essential or toxic elements are concerned, As, Ba, Cd, Cr(VI), Sn, Tl, Te, Ti, Ce, La, U were always < 0.326 mg/kg$_{ww}$, Th < 1.63 mg/kg$_{ww}$ and Hg < 0.0163 mg/kg$_{ww}$ The MDC for Cd (0.326 mg/kg $_{ww}$) is approximately 10-fold higher than maximum admissible level (0.05 mg/kg $_{ww}$) established by the European Commission (EC) in 2006, therefore pre-concentration techniques will be applied in further studies to reach lower detection limits. In any case, high contamination levels, if present, would also have been detected with these measurements. The EC has not established statutory limits for As and Hg for meat, but these elements were always <0.326 and < 0.0163 mg/kg $_{ww}$ respectively. Hg concentration, being < 0.0163 mg/kg$_{ww}$ for all samples, always resulted lower than 0.5 mg/kg $_{ww}$ allowed in fish (European Commission, 2006). The Al, Pb, Sb, Sr, Rb and Ni arithmetical mean and relevant standard deviation, minimum and maximum concentration are presented in Tables 5. For the mean concentration, the values below MDC not were considered. Al was detectable in 93% of samples and ranged from <0.326 to 10 mg/kg$_{ww}$ (mean concentration 3.6±3.1 mg/kg$_{ww}$). Ni and Sr were detectable in 21% of samples and ranged from <0.326 to 1.15 mg/kg$_{ww}$ (in sample 12, wood pigeon) and from <0.326 to 1.64 mg/kg$_{ww}$ (in sample 7, turtledove) respectively. Rb was detectable in 100% of samples and ranged from 1.34 to 25.3 mg/kg$_{ww}$ (mean concentration 7.77±6.19 mg/kg$_{ww}$).

Lead was detectable in 64% of samples (mean concentration 16.9±32.4 mg/kg$_{ww}$). Pb showed the maximum concentration in sample 13 (quail from Romania).When detectable, Pb concentrations always exceeded maximum admissible levels for Pb (0.1 mg/kg $_{ww}$) established by the Commission Regulation 1881/2006 (EC, 2006). The MDC for Pb (0.326 mg/kg $_{ww}$) is approximately 3-fold higher than maximum admissible level (0.1 mg/kg $_{ww}$) established by the EC in 2006, therefore pre-concentration techniques will be applied in further studies to reach lower detection limits. The Pb concentration resulted <0.326 mg/kg$_{ww}$ for 5 samples and ranged between 0.5 and 0.95 mg/kg$_{ww}$ for 3 samples, between 1 and 9 mg/kg$_{ww}$ for 4 samples and from

33 to 99 for 2 samples (samples N.7 and 13). Antimony resulted detectable only in the sample 13 (quail from Romania) showing in this sample a concentration of 1mg/kg$_{ww}$

The consumption of wild meat in Western countries showed an increase in recent years. Wild meat, in fact, are part of diet due to their high content of protein, fatty acid, vitamins and minerals. In order to assess beneficial health effects and risks due to the consumption of wild animal meat for Italian people, it was essential to know the amount of game consumed. Annual consumption of wild animal meat for an average Italian person is negligible. For the critical group of hunters, average annual consumption of wild animal meat is estimated to be 8 kg, and a single consumption of 150 g. The Population Reference Intake (PRI) and Adequate Intake (AI) (EFSA, 2006) were used in this study to evaluate the benefits from game consumption. Table 6 shows for some essential elements the mean and maximum concentration, daily intake (mg/day) from a 150g daily wild meat consumption and the comparison with nutritional requirements.

Among major elements, a game consumption of 150 g/day resulted in a daily intake of K (calculated from the mean and maximum concentrations respectively) of 552 and 839 mg/day, 231 and 287 mg/day of Na, 50 and 283 mg/day of calcium, 35 and 51 mg/day of Mg, 424 and 579 mg/day of P, 443 and 647 mg/day of S; for K and Na the daily dose represents the 6-14 and 15-19% respectively of AI, for Mg, Ca and P the 14-21, 5-28 and 61-81 % respectively of PRI.

Among trace elements, game consumption of 150 g/day accounted for a daily intake (calculated from the mean and maximum concentrations) of 6.6-10.8, 1.4-1.8, 0.47-0.65, and 0.07-0.16 mg/day of Fe, Zn, Cu and Mn respectively which represent the 52.2-83.3 % of AI for Cu, and the 66-108, 12-15 and 2.6-5.9% respectively of PRI for Fe, Zn and Mn.

DISCUSSION

The high Pb concentrations found in the samples are probably due to the presence of bullets in game meat. The different results can be explained by taking into account the size of the sample and that the distribution of lead ammunition fragments is not homogeneous within the animal shot. The individuals examined differ in the lead contents in their tissues surrounding the entry and exit wounds and at different sites along the bullet pathway. The heterogeneous distribution of Pb in tissue due to the use of lead bullets has been noted by several researchers (Tsuji, et al., 2009). The results show that bullet-derived lead in game meat is an important source of human contamination; careless removal of tissues from around the bullet pathway in the animal body results in elevated lead doses from meat ingested by humans. In fact, Pb bullets are known to fragment as they pass into and through the game tissues, and hundreds of tiny fragments may radiate into tissues up to distance of 45 cm from the bullet path (Taggart, et al., 2011, Dobrowolska and Melosik, 2008). This means that it is almost impossible to eliminate this source of food contamination, as long as Pb bullets remain in use. As the game is now being made increasingly available to the consumers *via* restaurants, supermarkets and high street butchers, and is often promoted as a healthy, wild alternative to otherwise often intensively farmed meat products, it is necessary to make due consideration of human and animal health risks.

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Table 1. Species, family and characteristics of the samples, year and site of the hunt,

N°	Sample	Species	Family	Characteristics	Hunting site (year)
1	Mallard or Wild duck	*Anas platyrhynchos*	Anatidae	Not passerine Migratory	Ungary (2012)
2	Pheasant	*Phasianus colchicus*	Phasianidae	Not passerine Not migratory	Central Italy (2012)
3	Woodcock	*Scolopax rusticola*	Scolopacidae	Not passerine Migratory	Central Italy (2013)
4	Woodcock	*Scolopax rusticola*	Scolopacidae	Not passerine Migratory	Central Italy (2013)
5	Wood pigeon	*Columba palumbus*	Columbidae	Not passerine Migratory	Central Italy (2013)
6	Trush	*Turdus philomelos*	Turdidae	Passerine Migratory	Central Italy (2013)
7	Turtledove	*Streptopelia turtur*	Columbidae	Not passerine Migratory	Central Italy (2013)
8	Woodcock	*Scolopax rusticola*	Scolopacidae	Not passerine Migratory	Sweden (Gotland) (2014)
9	Trush	*Turdus philomelos*	Turdidae	Passerine Migratory	Greece (2014)
10	Wood pigeon	*Columba palumbus*	Columbidae	Not passerine Migratory	Central Italy (2014)
11	Wood pigeon	*Columba palumbus*	Columbidae	Not passerine Migratory	Central Italy (2014)
12	Wood pigeon	*Columba palumbus*	Columbidae	Not passerine Migratory	Central Italy(2014)
13	Quail	*Coturnix coturnix*	Phasianidae	Not passerine Migratory	Romania(2014)
14	Woodcock	*Scolopax rusticola*	Scolopacidae	Not passerine Migratory	Croatia(2014)

Tabella 2: Elements and relevant method of determination, detection limit, MDC (number of the sample with concentration > MDC)

	Element	**MDC (mg/kg_w)**	**Method**
Major	Calcio	0.326 (14)	EPA 3050B 1996 + EPA6010D 2014
	Fosforo	0.326(14)	EPA 3050B 1996 + EPA6010D 2014
	Magnesio	0.326 (14)	EPA 3050B 1996 + EPA6010D 2014
	Sodio	0.326 (14)	EPA 3050B 1996 + EPA6010D 2014
	Potassio	0.326(14)	EPA 3050B 1996 + EPA6010D 2014
	Zolfo	0.326 (14)	EPA 3050B 1996 + EPA6010D 2014
Minor	Ferro	0.326 (14)	EPA 3050B 1996 + EPA6010D 2014
	Manganese	0.326 (12)	EPA 3050B 1996 + EPA6010D 2014
	Rame	0.326 (14)	EPA 3050B 1996 + EPA6010D 2014
	Silicio	0.326 (13)	EPA 3050B 1996 + EPA6010D 2014
	Zinco	0.326 (14)	EPA 3050B 1996 + EPA6010D 2014
	Cobalto	0.326 (0)	EPA 3050B 1996 + EPA6010D 2014
	Cromo (total)	0.326 (0)	EPA 3050B 1996 + EPA6010D 2014
Non essential or toxic	Alluminio	0.326 (13)	EPA 3050B 1996 + EPA6010D 2014
	Antimonio	0.326 (1)	EPA 3050B 1996 + EPA6010D 2014
	Nichel	0.326 (3)	EPA 3050B 1996 + EPA6010D 2014
	Piombo	0.326 (9)	EPA 3050B 1996 + EPA6010D 2014
	Stronzio	0.326 (3)	EPA 3050B 1996 + EPA6010D 2014
	Rubidio	0.652 (14)	EPA 3050B 1996 + EPA6020B 2014
	Arsenico	0.326 (0)	EPA 3050B 1996 + EPA6010D 2014
	Bario	0.326 (0)	EPA 3050B 1996 + EPA6010D 2014
	Cadmio	0.326 (0)	EPA 3050B 1996 + EPA6010D 2014
	Stagno	0.326 (0)	EPA 3050B 1996 + EPA6010D 2014
	Tallio	0.326 (0)	EPA 3050B 1996 + EPA6010D 2014
	Tellurio	0.326 (0)	EPA 3050B 1996 + EPA6010D 2014
	Titanio	0.326 (0)	EPA 3050B 1996 + EPA6010D 2014
	Torio	1.63 (0)	EPA 3050B 1996 + EPA6010D 2014
	Cerio	0.652 (0)	EPA 3050B 1996 + EPA6020B 2014

Lantanio	0.652 (0)	EPA 3050B 1996 + EPA6020B 2014
Uranio	0.652 (0)	EPA 3050B 1996 + EPA6020B 2014
Mercurio	0.0163 (0)	EPA 3050B 1996 + EPA 7470A 1994

Table 3

Concentration (mg/kg$_{ww}$) of essential (major) elements in wild bird samples

N°	Samples	Ca	P	Mg	Na	K	S
1	Wild duck	148	2061	179	1795	2175	2562
2	Pheasant	83	2380	232	1600	3460	2613
3	Woodcock	123	2961	245	1728	4302	3235
4	Woodcock	230	3861	339	1222	5591	4311
5	Wood pigeon	74	2817	233	1426	3604	2867
6	Trush	733	3473	259	1726	4134	3388
7	Turtledove	1890	2965	200	1023	1885	2643
8	Woodcock	148	2479	208	1167	3614	2851
9	Trush	183	2995	241	1562	4264	2863
10	Wood pigeon	57	2800	230	1917	3747	2875
11	Wood pigeon	71	2984	229	1850	3877	3090
12	Wood pigeon	80	2730	225	1812	3675	2531
13	Quail	811	2632	206	1438	3191	2693
14	Woodcock	55	2468	208	1329	3997	2788
	Minimum	55	2061	179	1023	1885	2531
	Maximum	1890	3861	339	1917	5591	4311
	Mean	335	2829	231	1542	3680	2951
	Stand. dev.	509	452	37	281	899	464

Table 4

Concentration (mg/kg$_{ww}$) of essential (minor or trace) elements in wild bird samples

N°	Samples	Fe	Mn	Cu	Si	Zn
1	Wild duck	46	0.27	4	2.71	12
2	Pheasant	13	< 0.326	1	2.08	10
3	Woodcock	39	0.30	3	1.52	9
4	Woodcock	45	< 0.326	4	1.34	12
5	Wood pigeon	36	0.29	3	0.86	10
6	Trush	72	0.66	5	1.32	11
7	Turtledove	48	0.55	3	2.19	10
8	Woodcock	27	0.31	2	0.63	8
9	Trush	49	0.56	4	0.85	7
10	Wood pigeon	46	0.29	4	< 0.326	10
11	Wood pigeon	64	0.30	4	1.82	10
12	Wood pigeon	39	0.58	3	0.58	8
13	Quail	60	1.04	2	7.63	10
14	Woodcock	35	0.26	2	1.06	6
	Minimum	13	< 0.326	1	< 0.326	6
	Maximum	72	1.04	5	7.63	12
	Mean	44	0.45	3	2	9
	Stand. dev.	15	0.24	1.10	1.84	1.74

Table 5
Concentration (mg/kg$_{ww}$) of not essential or toxic elements in wild bird samples

N°	Samples	Al	Sb	Ni	Pb	Sr	Rb
1	Wild duck	4	< 0.326	< 0.326	0.54	0.54	2.17
2	Pheasant	5	< 0.326	< 0.326	1.78	< 0.326	14.3
3	Woodcock	1	< 0.326	< 0.326	5.78	< 0.326	9.12
4	Woodcock	1	< 0.326	< 0.326	8.90	< 0.326	1.34
5	Wood pigeon	5	< 0.326	< 0.326	< 0.326	< 0.326	6.03
6	Trush	< 0.326	< 0.326	< 0.326	1.32	< 0.326	8.58
7	Turtledove	9	< 0.326	< 0.326	33.4	1.64	11.5
8	Woodcock	1	< 0.326	0.89	0.94	< 0.326	4.38
9	Trush	1	< 0.326	< 0.326	0.56	< 0.326	4.79
10	Wood pigeon	1	< 0.326	< 0.326	< 0.326	< 0.326	5.99
11	Wood pigeon	4	< 0.326	< 0.326	< 0.326	< 0.326	7.9
12	Wood pigeon	1	< 0.326	1.15	< 0.326	< 0.326	25.3
13	Quail	10	1	< 0.326	98.5	0.69	3.47
14	Woodcock	4	< 0.326	0.53	< 0.326	< 0.326	3.96
	Minimum	< 0.326	< 0.326	< 0.326	< 0.326	< 0.326	1.34
	Maximum	10	1.00	1.15	98.55	1.64	25
	Mean	3.6	1.00	0.86	16.9	0.96	7.77
	Stand.dev.	3.1		0.31	32.4	0.60	6.19

Table 6 Element mean (for the mean calculation, the values below MDC were not considered) and maximum concentration (mg/kg), relevant daily dose (mg/day) from wild birds consumption and comparison with daily dose risk estimators (PTWI= Provisional Tolerable Weekly Intake; UL= Tolerable Upper Intake Level) for a 70 kg man (EFSA, 2006; JECFA, 2006) and with nutritional requirements (PRI= Population reference intake; AI= adequate intake) (EFSA, 2006)

Element	Sample with a maximum concentration	Mean-max concentration	Daily dose (mg) for 150 g consumption	Daily dose from risk estimators	Daily nutritional requirements
Al	13	3.6-10	0.54-1.5	10 (from PTWI)	
Pb	13	16.9-98.5	2.5-14.8	0.250(from PTWI)	
Zn	1 and 4	9.5-12	1.4-1.8	25 (from UL)	12 (PRI)
Cu	6	3.14-5	0.47-0.75	5 (from UL)	0.9 (AI)
Fe	6	44-72	6.6-10.8		10 (PRI)
Mn	13	0.45-1.04	0.07-0.16		2.7 (PRI)
K	4	3680-5591	552-839		3900 (AI)
Na	10	1542-1917	231-287		1500(AI)
Mg	4	231-339	34.6-50.8		240 (PRI)
Ca	7	335-1890	50.2-283	2500 (from UL)	1000 (PRI)
P	4	2829-3861	424-569		700 (PRI)