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Relationships between stomach content and concentrations of essential and non-essential elements in tissues of omnivorous nestling rooks *Corvus frugilegus*: Is the size and composition of stomach content relevant?

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Abstract. Soil-invertebrate feeding birds can be exposed to high doses of toxic metals through their diet. Recently, we have shown that nestling rooks *Corvus frugilegus* from several rookeries in Poland have a cadmium (Cd) tissue level diagnostic for acute contamination as well as an elevated level of lead (Pb). To explain the potential pathway of bioaccumulation of 11 essential and non-essential elements, including two metals of primary concern (Cd and Pb), in target tissues of these nestlings, we analyzed the relationships between the dietary characteristics of stomach content (mass of digesta, number of cereal grains, plant and animal items, and grit particles) and concentrations of these elements determined in the liver, kidneys, lung, muscles and bones. Our analysis showed in total 17 (8 negative and 9 positive) statistically significant relationships between the five analyzed dietary characteristics of stomach content and concentrations of metals in the liver, kidneys, muscles and bone. We found a significant positive relationship between the number of animal food items and Cd-level in kidneys; and a negative relationship between the number of plant items and Pb-level in the liver, and between the number of grit particles and Pb-level in kidneys. Despite the limitations of our study due to the different degree of digestion of some food items, our findings suggest high bioavailability of Cd from animal food items and a low level or reduced gastrointestinal absorption of Pb from plant food (mainly cereals). We urge further research on absorption of Cd and Pb from different dietary components and application of diet analysis to explain the complex nature of bioaccumulation of anthropogenic contaminants in the internal organs and tissues of birds and other species of animals, especially in species with a mixed plant-animal diet.

Key words: non-essential metals, lead, cadmium, soil-invertebrate feeding birds, bird diet

Introduction

The dietary pathways of cadmium (Cd) and lead (Pb) are the main factors of bioaccumulation of these contaminants in wildlife (Andersen et al. 2004, Zhuang et al. 2009, Fritsch et al. 2012). While animal tissues are broadly used in monitoring studies of toxic substances (Rattner 2009), most results of ecotoxicological research are presented in a simplified manner, where only the concentrations or content of contaminants and comparisons between two or more sets of data (e.g. between contaminated *vs.* uncontaminated sites, various tissues, sexes, age classes) are presented. Considering the large variation in physiological and biochemical activity of different organs and tissues, such approaches to bioaccumulation of toxic substances mean that many

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interactions between contaminants and features of examined individuals and their environment are neglected (discussed in Fritsch et al. 2012). In spite of the huge number of papers on the accumulation of non-essential metals in wildlife, researchers have rarely linked the level of toxic metals or other contaminants to features of the environment, including the level of contaminants in various diet items (Lebedeva 1997, Fritsch et al. 2012, Schipper et al. 2012).

Birds that feed on soil invertebrates in agricultural land are especially likely to ingest high doses of non-essential elements, mainly cadmium (Pinowski et al. 1983, Carpene et al. 2006, Roodbergen et al. 2008, Hiller & Barclay 2011, Fritsch et al. 2012, Schipper et al. 2012). In the 20th century, agricultural activity caused an apparent increase of environmental pollution, especially in the concentration of non-essential elements and agrochemicals in arable topsoil. For instance, in Sweden's agricultural soils, cadmium levels increased by 0.03 % to 0.05 % per year (Olsson et al. 2005). The main sources of cadmium and lead in arable soils are organic fertilizers: both farmyard manure and slurry contain residues of feed components with extremely high content of this metal (Linden et al. 2001).

Recently, we found that nestling rooks *Corvus frugilegus* – a typical agricultural bird species for European lowland farmland – from several breeding colonies have cadmium tissue levels diagnostic for acute contamination, and elevated levels of lead (Orłowski et al. 2012a). In the present paper, we ask whether the diet composition determined post-mortem (the main dietary items identified in the stomach contents) is related to the level of essential and non-essential elements, and especially what dietary items might be responsible for the high levels of cadmium and lead found in these nestlings. Nestling rooks have mixed diets based both on plant and animal material gathered on arable land (Kasprzykowski 2003, Orłowski et al. 2009), where the level of non-essential elements and agrochemicals could be extremely high (Linden et al. 2001, Fritsch et al. 2012, Schipper et al. 2012). Hence, we predicted that both mass of whole stomach content and each of three identified major food components (plant, animal and mineral) will influence the bioaccumulation of metals. For example, the number of different plant (mainly cereal grain) and animal (mainly ground-dwelling Coleoptera) items (see Table 1 for stomach content composition) may increase the load of contaminants. On the other hand, mineral items (grit) containing calcium (e.g. fragments of bricks, shells of bird eggs or soil) may diminish the negative impact of non-essential elements or toxic metals (Sheppard et al. 1995, Hui 2004). Considering the critical role of the kidneys and liver in cadmium detoxification, resulting from their role in the transport of nutrients, we predicted that the impact of diet on the pollution level may be the strongest in these organs, in comparison to other analyzed tissues (Scheuhammer 1996, reviewed in Burger 2008). Recently, metal levels were assessed in different food items and stomach contents of soil-invertebrate feeding birds (Berglund et al. 2010, Fritsch et al. 2012, Schipper et al. 2012). However to our knowledge, this paper is the first attempt to apply the quantitative description of different stomach content components as potential factors explaining the concentration of some minerals and toxic metals.

The key objective of the presented work is to assess the relationships between the numerical characteristics of stomach content and concentrations of essential and non-essential elements, including two metals of primary concern (Pb and Cd), assessed in five tissues of omnivorous nestling rooks (Orłowski et al. 2012a). The results of this analysis will be useful in explaining the acute Cd contamination and elevated level of Pb that characterized previously examined nestlings (Orłowski et al. 2012a). In the previous study, the tissue concentrations of non-essential elements were not correlated with the age of the examined nestlings (Orłowski et al. 2012a). Finally, due to the different

Table 1. The quantitative characteristics of three groups of diet components found in stomach contents of 35 rooks *Corvus frugilegus* nestlings subjected to analysis of essential and trace metals. [1]Coleoptera included four families: Curculionidae, Carabidae, Elateridae and Scarabeidae; larv = larvae, im = imagi.

Component (total number of identified items)	Description or taxonomical characteristics (number of items)	Number of chicks with given component	Number of items in one nestling	
			median	min-max
Animal (224)	[1]Coleoptera: larv (96), im (117); Hymenoptera: im (2), Orthoptera: im (1), Neuroptera: im (1), Lepidoptera: caterpillar (5), Diptera: im (1), Heteroptera: im (1)	27	2	1-50
Plant (60)	Cereal grain: rye (40), oat (2), wheat (4), weed seeds (2), stems and leaves (7), plant roots (2), stems of moss (3)	16	2	1-20
Mineral (239)	Fragments of bricks (76), small stones (158), shells of bird eggs (5)	24	5	1-60

Table 2. Tissue concentration of different elements (mg/kg dry weight; ppm) in dead nestlings (1-13 days old) of the rook *Corvus frugilegus* from seven breeding colonies in north-eastern Poland.

Tissue	Value	Chemical element										
		Na	K	Ca	Mg	Fe	Cu	Zn	Mn	Co	Cd	Pb
Liver	average	702.5	35.1	971.2	93.3	65.7	3.1	3.3	2.0	4.2	17.2	5.0
	median	629.5	24.2	324.7	70.0	63.0	1.7	1.4	1.5	4.1	17.2	6.4
	min-max	129.7-1864.0	10.7-99.5	206.0-8160.0	4.7-553.7	57.1-92.6	0.1-31.8	0.4-30.9	0.3-3.7	3.6-4.9	16.3-17.8	0.1-7.2
Lung	average	749.1	35.0	254.6	70.1	60.7	2.1	0.8	1.9	4.1	17.2	6.0
	median	555.8	20.2	229.3	42.7	59.3	1.7	0.6	1.5	4.1	17.2	6.6
	min-max	119.6-1864.0	10.8-99.4	178.1-594.2	2.4-323.1	57.0-83.7	0.2-4.6	0.3-3.4	0.9-7.1	3.6-4.9	15.6-17.8	0.9-7.4
Kidney	average	979.6	25.7	1133.6	95.6	60.0	2.1	1.6	2.5	4.1	17.0	5.1
	median	813.7	20.2	261.4	95.0	58.1	1.1	0.7	1.5	4.1	17.1	6.3
	min-max	36.8-1864.0	11.2-99.5	161.7-7978.0	2.7-366.5	56.8-96.8	0.1-13.8	0.2-10.8	0.3-7.1	3.6-4.9	15.5-17.7	0.4-7.2
Muscle	average	788.6	32.3	377.1	100.3	59.9	2.3	1.3	1.8	4.1	17.2	6.2
	median	479.7	22.6	246.5	88.0	59.5	2.6	0.7	1.5	4.0	17.2	6.6
	min-max	57.3-1864.0	10.3-99.0	147.8-4546.0	5.3-367.8	57.0-66.4	0.03-4.5	0.3-13.6	1.0-7.1	3..6-4.5	15.7-18.8	0.05-7.2
Bone	average	790.5	36.6	1332.7	119.3	59.3	2.1	1.2	1.9	4.1	17.2	6.0
	median	639.9	22.4	463.4	85.4	58.5	2.2	0.9	1.5	4.0	17.2	6.5
	min-max	13.3-1864.0	10.9-99.3	206.7-9817.0	8.1-589.9	56.5-70.6	0.1-4.5	0.3-5.1	1.5-3.6	3.6-4.5	16.5-17.7	2.5-7.6

degree of digestion of some food items and post-mortem changes, we discuss the potential limitations of ecotoxicological studies using dead animals.

Material and Methods

Nestlings, food composition and metal analysis

In the study we used data on concentrations of 11 elements (Na, K, Ca, Mg, Fe, Cu, Zn, Mn, Co, Cd, Pb) determined in thirty-five rook nestlings aged from 1 to 13 days (Table 2). The nestlings were collected in seven breeding colonies located in north-eastern Poland within 50 km of Siedlce town (52°12′ N, 22°07′ E) at the following sites (villages): Ceranów, Podnieśno, Kotuń, Mokobody, Mordy, Stoczek and Siedlce. Nestlings were collected from 27 April to 7 May 2005. On the day of collection, all nestlings were stored frozen at –25 °C until the time of further analyses. Detailed results on metal levels in nestling tissues were presented in an earlier paper (Orłowski et al. 2012a). We determined the age of sampled nestlings using the growth curves of 97 individually-marked nestlings monitored during post-hatching development (Orłowski et al. 2012a); a brief synopsis of methods used in this study follows.

The adult rooks from studied colonies feed mainly on spring-sown cereals and grasslands (Kasprzykowski 2003). The food composition in sampled nestlings is based on analysis of stomach content; a detailed description of this analysis was presented elsewhere (Orłowski et al. 2009). The gizzard was cut off just above the esophagus and below the small intestine. Soon afterwards, the stomach contents were extracted, weighed and placed in 70 % alcohol. The food items in examined nestling were divided into three categories: animal, plant and mineral. Stomach content was found in 32 of 35 nestlings. In individual nestlings, the weight of stomach content ranged from 0.02 to 8.56 g (average = 1.44 g, median = 0.52 g). In total, across all nestlings we found 523 food items (Table 1).

Chemical elements analyses demanded the whole selected organs and tissues. Thus, we separated five organs and tissues: 1) liver, 2) kidneys, 3) lung, 4) pectoral muscles (hereafter "muscles") and 5) femur ("bones"). Samples for chemical analyses (5-151 mg of dry weight of each tissue) were weighed and dried to a constant mass at temperature of 65 °C. Samples were then homogenized using a porcelain pestle and mortar. Analyses were conducted with the use of an atomic absorption spectrophotometer (AAS; Weltz 1985) by Perkin-Elmer (AAnalyst 800-RW0683/3PYC; PerkinElmer Life and Analytical Sciences, Shelton). Standard curves were prepared using standardized samples (Merk). Concentrations of elements, were given in terms of mg kg^{-1} dry weight (dw) with an accuracy of 1 decimal point. Element determination was preceded by mineralization of samples, which was performed using a Berghof Speedwave MWS-2 system (microwave pressure digestion unit with built-in in situ temperature measurement; Berghof, Products Instruments GmbH, Eningen, Germany) and 65 % pure nitric acid (Sigma-Aldrich) for analysis. This involved the following procedure: samples (5-151 mg dw) were weighed and placed in calibrated 25 mL test tubes that were washed and rinsed with double-distilled water. According to the manufacturer's standard procedures, analytically pure concentrated nitric acid (1.5 mL 65 %; Sigma-Aldrich) was added to each sample to obtain clear solution, and the tubes were left to stand at room temperature for 20-24 h. Perchloric acid (0.5 mL 62 %) was then added to the contents of each tube and mixed before being placed in a mineralizer (Berghof Speedwave MWS-2 system) that was electrically heated to 400 °C and fitted with a regulator and temperature gauge (microwave pressure digestion unit with built-in in situ temperature measurement). The mixtures were heated at 100 °C for 1 h, at 150 °C for another hour, and then at 200 °C for a final hour (according to standardized method given by the system's manufacturer) until approximately 0.2 mL of colourless, clear solution was obtained. After cooling, each mixture was filled with double-distilled water to a volume of 5 ml, stirred, and then poured in its entirety into tightly sealable and absolute neutral polyethylene Nalgene containers (Nalge Nunc) according to the manufacturer's standardized method. In each case, all glassware and plastic containers were washed in 65 % pure nitric acid solution (Sigma-Aldrich) before analysis to remove the adsorbed metals. We used reference materials for each of the AAS analytical measurements (for more details on chemical analysis see: Orłowski et al. 2012a).

Data analysis

We predicted that the concentration of metals in analyzed tissues will be related both to the weight of stomach content and the number of major food categories, namely cereal grains, plant items, animal items and grit particles (Table 1; for the correlation

Table 3. Spearman rank correlation coefficients between the main components of stomach content and concentrations of metals in five tissues of nestlings rook *Corvus frugilegus* in various age, 1-13 days old. Asterisks denote the significance level: *p ≤ 0.05, **p < 0.01, ***p < 0.001; significant relationships are in bold.

Variable (characteristic)	Metal										
	Na	K	Ca	Mg	Fe	Cu	Zn	Mn	Co	Cd	Pb
Liver											
Age of nestlings	0.005	0.104	0.109	–0.287	0.293	–0.066	**0.404***	–0.047	0.022	0.018	–0.091
Digesta mass	–0.007	0.281	0.245	–0.074	–0.025	–0.167	–0.048	–0.162	0.170	0.187	–0.053
Number of cereal grains	0.133	0.354	–0.063	–0.227	0.309	0.412	0.342	**–0.565***	0.049	–0.247	**–0.600****
Number of plant food items	0.437	0.396	–0.164	–0.087	0.348	0.304	–0.026	–0.391	–0.081	–0.407	**–0.525***
Number of animal food items	0.086	0.096	–0.136	–0.326	0.110	0.000	0.143	–0.183	–0.006	–0.159	–0.160
Number of grit particles	0.257	–0.021	0.261	0.050	–0.027	–0.212	0.124	0.130	0.236	0.179	–0.013
Lung											
Age of nestlings	–0.003	0.123	0.229	0.017	**0.520****	0.037	**0.526****	–0.223	0.227	0.103	–0.069
Digesta mass	–0.155	0.245	0.281	0.122	–0.019	0.281	0.198	–0.205	0.040	0.184	–0.140
Number of cereal grains	–0.135	0.247	–0.143	0.011	0.250	0.386	0.372	–0.304	0.472	0.435	–0.225
Number of plant food items	0.172	0.079	–0.231	0.291	–0.158	0.421	–0.205	–0.357	0.181	0.343	–0.075
Number of animal food items	–0.017	–0.019	–0.028	–0.033	0.342	–0.083	0.151	–0.036	0.020	–0.031	0.174
Number of grit particles	–0.159	0.171	0.099	0.065	0.108	–0.091	0.314	–0.153	0.053	0.192	–0.008
Kidney											
Age of nestlings	0.088	–0.006	0.132	0.138	0.191	0.039	0.137	–0.266	0.286	–0.168	–0.202
Digesta mass	0.072	0.302	0.279	0.149	0.052	0.149	0.046	0.059	**0.433***	0.003	–0.257
Number of cereal grains	0.034	0.203	–0.269	–0.017	0.279	0.051	–0.100	–0.372	0.086	–0.014	0.027
Number of plant food items	0.094	0.262	–0.172	–0.100	–0.049	–0.095	–0.015	–0.179	0.092	0.121	0.313
Number of animal food items	0.278	–0.007	–0.220	–0.021	–0.216	0.093	–0.003	–0.147	**0.493***	**0.462***	0.098
Number of grit particles	–0.028	0.132	**0.392***	0.146	0.105	0.076	–0.002	0.285	**0.400***	–0.072	**–0.489****
Muscle											
Age of nestlings	–0.209	0.037	–0.046	0.173	0.306	–0.059	**0.439****	0.217	–0.083	0.199	–0.064
Digesta mass	0.007	0.230	0.245	**0.503****	**–0.352***	0.316	–0.124	0.195	–0.056	0.008	–0.197
Number of cereal grains	–0.113	**0.674****	–0.229	0.258	0.037	0.353	0.109	–0.062	–0.287	0.019	–0.014
Number of plant food items	–0.064	**0.487***	0.222	0.148	–0.229	–0.062	0.191	–0.183	–0.102	–0.081	–0.119
Number of animal food items	–0.186	–0.165	0.069	0.234	–0.012	0.098	–0.127	0.293	0.301	0.089	–0.130
Number of grit particles	–0.047	0.100	–0.073	0.323	–0.161	0.038	–0.095	0.212	–0.161	–0.034	–0.008
Bone											
Age of nestlings	–0.106	–0.079	**0.534*****	0.157	0.297	0.044	**0.369***	–0.113	–0.103	0.058	0.226
Digesta mass	**–0.368***	–0.033	0.295	–0.027	0.270	0.103	**0.411***	0.156	–0.247	0.170	0.025
Number of cereal grains	**–0.560***	0.333	0.355	0.098	0.455	0.080	0.458	–0.188	–0.138	0.120	0.382
Number of plant food items	**–0.675****	0.410	–0.069	–0.025	0.199	0.127	0.029	–0.077	–0.347	–0.299	0.414
Number of animal food items	–0.189	–0.188	0.137	–0.029	0.072	0.152	0.049	–0.058	0.025	–0.378	–0.015
Number of grit particles	–0.063	–0.066	0.116	–0.122	0.203	0.125	0.059	0.111	–0.039	0.264	–0.101

between concentrations of analyzed metals see: Orłowski et al. 2012b). Given the potentially high digestion of some food items, and lack of identified items (assigned to the category of plant/animal), this analysis took into account only nestlings with established presence of one of these components (= food positive nestlings). As a consequence of this approach, correlation analysis was performed for 16 and 27 food positive nestlings, with plant and animal food, respectively (Table 1). We found cereal grains in only six chicks. However, due to the presence of undigested cereal grains both in 1-2 days old nestlings (up to 4 seeds in one nestling) and older nestlings, 3-13-days-old (up to 20 seeds), the youngest nestlings (1-2-days-old) without the food were assigned a value of zero. After this procedure, a total of 18 nestlings were included in the correlation analysis for the cereal grains. A similar procedure was applied to mineral items (found in 24 nestlings), but in this case all the other nestlings, in which there was no grit particles (11 nestlings) were assigned a value of zero.

Initially, the dietary data and concentrations of metals were tested for goodness-of-fit to a normal distribution with the use of the Kolmogorov-Smirnov test. Since some data, both raw and log-transformed, had non-normal distribution (mainly dietary characteristics of stomach content and concentrations of some elements (Na, Ca or Pb)), we applied the non-parametric Spearman rank correlation coefficient (r_s) to assess the relationship between these two sets. To evaluate the potential explanations of findings, we presented the relationships between age and concentrations of metals, and various characteristic of stomach content of examined nestlings both in the main analysis (Table 3) and in Appendix. Statistical analyses of data were carried out with an aid of Excel and Statistica v.7.1 (Statsoft 2006) software.

Results

Our analysis showed in total 17 (including 8 negative and 9 positive) statistically significant relationships between the five analyzed dietary characteristics of stomach content (Table 1) and concentrations of metals (Table 2) for four (liver, kidneys, muscles and bone) of the five (with the exception of lung) examined tissues (Table 3).

In the liver, we found a negative relationship between the number of cereal grains and the level of Mn and Pb, and between the number of plant food items and Pb-level (Table 3).

In the kidneys, we found six significant relationships: positive relationships were between digesta mass and Co-level, between the number of animal food items and the concentration of Co and Cd, and between the number of grit particles and the concentration of Ca and Co, while the negative relationship was between the number of grit particles and the concentration of Pb. In muscles, digesta mass was positively correlated with Mg-level and negatively with Fe-level. The number of cereal grains and number of plant food items was positively correlated with concentration of K in muscle tissue.

In bone, Na-level was negatively correlated with digesta mass and number of cereal grains and plant food items; digesta mass was positively correlated with bone Zn concentration (Table 3).

Discussion

Our study describes the relationships between stomach contents and concentrations of minerals and metals, including the toxic Cd and Pb, in the internal organs, muscles and bones of bird nestlings. Our findings suggest that, in omnivorous species, food chain transfer of metals takes place via the animal fraction of the diet (primarily invertebrates) rather than plant material. Although we found variation in tissue responses to different dietary characteristics of the stomach contents, our study was limited by the different degree of digestion of some food items, and future work is needed to more fully understand diet-dependent bioaccumulation of metals. However, our study succeeded in two particularly important relationships: the positive relationship between the number of animal food items and Cd-level in kidneys, and the negative relationship between the number of plant items and Pb-level in the liver. These findings will be useful in assessing the risk and exposure to environmental contaminations of birds based on diet composition. Our findings are also important for understanding the complex nature of toxic metal bioaccumulation in animal organs and tissues, confirming to a large degree the findings of recent studies on contaminant flux through "food-chain effects" in terrestrial birds (c.f. Fritsch et al. 2012, Schipper et al. 2012).

As we expected, a statistically significant effect of dietary variables on cadmium concentration was detected only in the kidneys, which confirms the crucial role of this organ in the detoxification of cadmium (Mochizuki et al. 2008). Importantly, in spite of many earlier papers indicating a link between diet and cadmium contamination in bird kidneys and liver (Wenzel et al. 1996), to our knowledge, our findings expressed these relationships in a direct quantitative manner for the first time. Furthermore, our results showed a similar direction of relationship for Co (Table 3). In the environment, Co is synergistically related to Cd and Pb (Nagy & Konya 1998, Kalavrouziotis et al. 2009); we observed analogous relationships between these two metals in the kidneys and bone of examined nestlings (Orłowski et al. 2012b). Hence, we believe that our results may support earlier findings of high bioavailability of Cd and Co from animal food items, i.e. a high-protein diet (Kimura et al. 1998, reviewed in Asagba 2009). This most probably reflects overall high concentrations of these two metals in animal food components, mainly ground-dwelling invertebrates (Walker et al. 2002, Purchart & Kula 2007, Schipper et al. 2012). Lind et al. (1997) postulated that Cd from an occasional high Cd dose may be more readily absorbed because the Cd may induce non-specific damage to the intestinal mucosa, saturate the capacity of the intestinal mucosa to bind Cd, or alter permeability of tight-junctions between mucosal cells.

The negative relationship between the number of plant items and cereal grains and liver Pb concentrations (Table 3) may suggest a low level and/or reduced gastrointestinal absorption of Pb from plant food (mainly cereals), that is, food containing plant fiber and phytate (Lind et al. 1998). Additionally, we found that the liver Pb concentration in nestlings with cereal grain in their stomach contents was significantly lower than in nestlings without these food items (average = 2.93 *vs.* 5.48 ppm dw Pb, respectively; General Linear Model, F = 6.99, P = 0.012). According to House et al. (2003), the bioavailability of elements from plant foods depends on a complex set of interacting factors, including meal composition, the form of the element in the food, presence of putative antinutrients (e.g. phytate) that may suppress mineral absorption, and the physiological and nutritional status of the individual. The rook eats a variety of foods, and the importance of a specific food as a source for minerals may be different when the food is consumed as part of a complex diet than when it is consumed separately. Furthermore, the significant negative relationship we found between the number of grit particles and kidney Pb concentration (Table 3) suggests that these calcium-rich items could reduce the bioaccumulation of Pb (Eeva & Lehikoinen 2004, Dauwe et al. 2006).

The most unexpected findings in our study concern the relationships detected in muscles (K, Mg and Fe) and bones (Na and Zn). We believe that these findings may be partly the result of impaired balance of these metals resulting from weak physical condition of examined nestlings. On the other hand, because these relationships do not prove a causal relation with the diet, an alternative explanation may be postmortem processes (e.g. autolysis) that are associated with tissue swelling and destruction of cellular structures. However, of the elements analyzed in our study, only Na concentration may increase with latency (measured in human heart-muscle up to 50 hours) of post-mortem autolysis (Aalbers et al. 1987). Furthermore, intracellular elements and protein-bound trace elements are not vulnerable to autolysis (Aalbers et al. 1987). One may speculate that postmortem processes include decomposition of the digestive system along with their content, resulting in an additional factor influencing the level of some elements in adjacent tissues, especially in species with small body size (like some birds or nestlings). Dead animals are broadly used in ecotoxicological studies, since they are usually the only specimens available; a key future goal is developing methods of measuring metal bioaccumulation and toxicity in living individuals (Lavery et al. 2009).

Conclusions

Although our analyses are correlative and cannot demonstrate causal relationships, our findings concerning the toxic metals Cd and Pb in particular are consistent with earlier results, including experimental studies of the relationships between diet composition and the transfer of metals through the food-web. Our results are a promising attempt to quantitatively analyze stomach contents as potential predictors of toxic metals levels in the liver and kidneys; however, validating this approach would require additional analysis. The proportion of the diet that is based on plant versus animal food items is probably the main factor influencing the level of cadmium and lead in birds, including nestlings, with a mixed diet. On the other hand, the level of Cd and Pb in the organs and tissues that have lesser involvement with food digestion or detoxication (lung, muscles, bone) are most likely more dependent on the interaction of minerals and other trace metals. Considering that some nestlings analysed were of very young age (including nestlings of only 1 day old), it seems implausible that the Cd or Pb tissue concentrations result from food chain transfer, hence maternal transfer of this non-essential element seems a more likely explanation (c.f. Dauwe et al. 2005, Guirlet et al. 2008). Increasing levels of inorganic contaminants of anthropogenic origin in soils implies the urgent need for further research on the transfer of

Cd and Zn followed through a food chain consisting of plant and animal food items consumed by birds. We urge further research on absorption of Cd and Pb from different dietary components and application of diet analysis to explain the complex nature of bioaccumulation of anthropogenic contaminants in the internal organs and tissues of birds and other species of animals, especially in species with mixed plant-animal diets.

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Appendix. Spearman rank correlation coefficients between age and characteristics of stomach contents identified in 35 nestling rooks *Corvus frugilegus*. Asterisks denote the significance level: *p ≤ 0.05, **p < 0.01, ***p < 0.001; significant relationships are in bold.

Variable (characteristic)	Age of nestlings	Digesta mass	Number of cereal grains	Number of plant food items	Number of animal food items
Digesta mass	0.260	-		-	-
Number of cereal grains	**0.611****	**0.808*****	-	-	-
Number of plant food items	0.204	**0.578****	**0.919*****	-	-
Number of animal food items	**0.430***	**0.411***	0.288	0.360	-
Number of grit particles	0.218	**0.491***	–0.202	0.192	0.122