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ScienceDirect
International Journal of Mass Spectrometry 267 (2007) 109–116
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

Determination of trace elements in pigeon and raven feathers by ICPMS

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Received 27 October 2006; received in revised form 14 January 2007; accepted 20 February 2007
Available online 23 February 2007

Abstract

The concentration of 11 trace elements (Mg, Al, Mn, Cu, Zn, Rb, Mo, Cd, Ba, Hg and Pb) and sulfur in feathers of pigeons and ravens was determined by ICPMS after wet digestion of the sample. Pigeon feathers were collected from five habitats: rural, industrial, urban, natural area and from a controlled environment, and raven feathers from the former two habitats. The distribution along the feather shaft was studied and for most elements it was found that the concentration increased from the quill towards the distal end of the feather. There were statistically significant differences in the concentration of trace elements between pigeons from different environments and between ravens and pigeons from similar habitats. It was found that for most elements the lowest concentration was in feathers from the control population and the highest in the industrial habitat. In general, higher concentrations were found in the omnivore raven feathers than in the granivore pigeons. Sulfur, that is abundant in keratin, was found to be a potential internal standard as its concentration presented the lowest variation among different samples. Thus, feathers from common birds, collected from the ground, can serve as bio-indicators of environmental levels of trace elements.

Keywords: Trace elements; Environmental pollution; Feather; Bio-indicator; ICPMS

1. Introduction

Heavy metals are considered to be an important threat to the environment as they can accumulate within an ecosystem and endanger its health. However, unlike other sources that may cause population decline through individual mortality (e.g., hunting, poisoning, etc.) that are easy to detect, elevated levels of trace elements can affect many fitness components without resulting in immediate death. For that reason heavy metals can be considered, except in extreme situations of pollution, as a source of silent death that is extremely difficult to detect. Monitoring programs should be executed in order to identify elevated levels of toxic elements and to minimize their effect on the ecosystem. Most programs test for heavy metal accumulation in soil or water, however, the advantages of using biomonitors were recently acknowledged and an increasing number of monitoring programs now use plants or animals. Since plants are sessile and accumulate heavy metals from their surroundings they can be used to monitor the long-term dynamics of the pollution in a localized area. However to identify pollution in a larger area other bioindicators that reflect a coarser spatial scale should be used.

Birds use different sources of food and water in a relatively large area and thus the level of trace elements in birds' organs and feathers may reveal the levels of toxic elements in their entire home range. A very detailed review of birds as a means of bio-monitoring was published by Burger [1] and a recent summary of stable isotopes studies in bird collections was reported by Rocque [2]. Several studies focused on internal organs, such as liver, bone, brain, etc. for identification of environmental pollutants [3–4]. These usually required capturing the birds and sacrificing them. Eggs and eggshells have also been used to evaluate the effect of environmental pollutants [5]. The use of feathers, whether collected from the ground under the birds' nesting areas and habitats, or whether collected from birds that are captured and then released, makes such studies much easier [6–10]. Similarly, using common species with wide distributions prevents undesirable effects on an endangered species population and may allow comparing levels of trace elements accumulation between different environments.

In the present study feathers of two common species of resident birds the Hooded Crow (*Corvus corone cornix*) and the

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doi:10.1016/j.ijms.2007.02.022

Rock Dove/Feral Pigeon (*Columba livia*) were examined as bioindicators for trace elements pollution. The focus was on arid environments affected by different anthropogenic land uses: industrial, urban, rural and natural areas. Both species are very common in many habitats across the Negev desert in Israel and therefore can be good candidates for comparative studies. Moreover, different diets of pigeons and ravens (i.e., granivores and omnivores, respectively) may reveal different sources of pollution. By monitoring both species simultaneously the spectrum of potential pollution sources may be expanded and differences between the species may improve the ability to identify possible contamination sources. Except for using the species as bioindicators for environmental pollution focusing on a common granivorous bird (i.e., pigeon) may reveal possible threats to other populations of endangered granivorous species. For example, four endangered species of Sandgrouses (*Pterocles*) in the Negev desert suffered severe population reduction in the last two decades without any noticeable cause. Finding elevated levels of toxic elements in pigeon feathers may point to similar problems in Sandgrouses without disturbing their declining populations.

Many of the reports on the level of toxic elements in feathers dealt with mercury and arsenic, especially when these studies involved seabirds, such as gulls or cormorants [8–10]. Different analytical techniques were used in these studies including inductively coupled plasma mass spectrometry (ICPMS) [3,4,6,9] and laser ablation-ICPMS [9], inductively coupled plasma atomic emission spectrometry (ICP-AES) [10] as well as graphite-furnace atomic absorption spectroscopy (GF-AAS) [7]. Imaging of copper, zinc, iron, phosphorus and sulfur in biological tissues, although not feathers, was recently carried out by the powerful technique of LA-ICPMS [11–14] and MALDI-FTICR-MS [11]. This technique has the potential to provide a more detailed picture of elemental distribution in the feather than obtained by other techniques.

In the present study the concentration of eleven trace elements (Mg, Al, Mn, Cu, Zn, Rb, Mo, Cd, Ba, Hg and Pb) and of sulfur that is a minor component, were determined in feathers. These can be divided, somewhat arbitrarily, into three groups: essential elements (Mg, Al, Mn, Cu, Zn and S), toxic elements (Cd, Pb and Hg) and other elements that are neither toxic at low levels nor considered as truly essential elements (Rb, Mo and Ba). The analytes selected for the present study did not include other important elements like Cr, Fe, Se and As that could be problematic for ICP-quadrupole MS studies in biological matrices or known toxic elements like Tl and U that are present at very low levels. The analytical method used in the present study was based on removal of external contamination from the feathers by rinsing and then wet digestion of the feathers followed by ICPMS analysis of the liquid samples.

The main objective of this study was to examine whether feathers of common birds that were collected from the ground and analyzed by means of wet digestion followed by ICPMS analysis can reveal elevated levels of trace elements in the environment. To achieve this goal, candidate elements for internal standard were examined and the use of different feathers and feather segments were compared. Then, the proposed methodology (distal part of primary feathers) was examined by comparing trace element levels in feathers of pigeons living in the five different habitats and between granivores (pigeons) and omnivores (ravens) in two different environments. Birds from the industrial environment are expected to contain higher levels of toxic and other trace elements than birds from the control and natural environments. Omnivores are expected to contain higher levels of trace elements than granivore birds since they are positioned higher in the food web.

2. Experimental

2.1. Sample collection

Pigeon feathers were collected from four different locations characterized by different anthropogenic land uses in the Negev desert, Israel. Additionally, pigeon feathers from a captive population with known dietary habits were added to serve as a control. Samples were collected from an urban environment in the city of Beer-Sheva, from a rural area in the Nahal Ashan farm, from an industrial environment and from the open natural environment of Borot Lotz. In each location ten feathers were collected with special care to have them well separated from each other to avoid pseudoreplication.

The raven feathers were gathered in the same industrial area as the pigeons and from a rural area near Kibbutz Urim and Kibbutz Zeelim. The rural ravens were culled as part of a culling operation executed by the Israeli Nature and Parks Authority. This made it possible to compare trace elements concentrations between different kinds of feathers and to compare these feathers to the trace element content in talons of the same individual.

2.2. Sample treatment and analysis

The feathers were washed in order to remove external contamination, according to the procedure developed for human hair and nails samples [15–16]. The feathers were cut into sections according to the distance from the bottom of the shaft, and samples weighing between 30 and 100 mg were placed in a 15 ml capped polystyrene test tube. Each sample was first rinsed with doubly distilled water (DDW) with 1 g l^{-1} Triton X-100 detergent (Merck, Darmstadt, Germany) for 15 min in an ultrasonic bath. The liquid was carefully drained from the test tube and the procedure was repeated twice with DDW, then with acetone and finally once more with DDW.

Digestion of the sample was carried out by carefully adding 2 ml of high purity concentrated nitric acid (Suprapur, Merck, Darmstadt, Germany) and 1 ml of 30% hydrogen peroxide (Merck) to each test-tube. The test-tubes were then placed in a hot water bath, at a close to boiling temperature (caution: the caps must be loosely screwed to prevent the build-up of gases that evolve in the digestion process). After about 60 min the sample was completely digested in most cases. If a visible precipitate remained another portion of the digesting solution was added for a second round. Each set of 15 samples was accompanied by a blank test-tube that served as the series blank. Rhodium was added as an internal standard to all test-tubes, so that its final concentration was 10 ppb (10 μg l^{-1}). The digested samples are then diluted with DDW to a volume of 15 ml and analyzed. The raven talons were treated in a similar fashion.

Analysis was carried out by an inductively coupled plasma mass spectrometer (ICPMS), (Elan-6000, Sciex/Perkin-Elmer, Thornhill, Canada) at the Geological Survey for Israel (GSI), in Jerusalem. The instrument was calibrated with a multi-element standard solution ICP-VI (Merck, Darmstadt, Germany). Calibration for mercury was carried out with a freshly prepared 10 μg l^{-1} standard in a solution of potassium bromide and potassium bromate stored in a dark glass vial in order to prevent decomposition. Sulfur calibration was carried out with a solution of 100 mg l^{-1} sulfate in sodium sulfate (elemental sulfur was 33.4 mg l^{-1}). Analysis included the injection of the sample for 25 s (about 400 μl) and measurement of the following ions: ^{26}Mg, ^{27}Al, ^{34}S, ^{55}Mn, ^{63}Cu, ^{64}Zn, ^{85}Rb, ^{98}Mo, ^{114}Cd, ^{138}Ba, ^{202}Hg, ^{208}Pb and ^{103}Rh. The peak area was calculated after subtraction of the baseline and the concentration of each element was calculated relative to the peak area of the standard solution measured under the same conditions. Due to the high content of magnesium and sulfur in the feathers the minor isotopes (^{26}Mg and ^{34}S) were selected in order to avoid detector saturation during the measurement.

After measurement of all the analytes, the results were corrected for the matrix effect by comparing the rhodium signal of the blank samples to that of the sample. The corrected concentration and weight of the sample were then used to calculate the level of the trace element in the original feather section.

2.3. Data analysis

The data was checked for all relevant assumptions before performing statistical analysis [17]. Some variables were log transformed to normalize the variance and to achieve homogeneity of the variance.

To reveal the overall variation in the concentration of each element the coefficient of variation (CV) was calculated using all samples of upper shaft of both pigeons and ravens from all populations ($n = 69$). The coefficient of variation allows comparison of the degree of variation between concentrations of different elements that differ drastically in their mean concentration. Because sulfur presented the lowest coefficient of variation four more statistical analyses were performed to examine whether sulfur is suitable to serve as an internal standard for measurements of trace elements in feathers. One-way repeated measure ANOVA was performed followed by pairwise comparison to determine the distribution of sulfur along the pigeon feather shaft. To compare the sulfur concentration in feathers (upper-shaft) between pigeons and ravens a non-parametric Mann–Whitney U-test was used. A non-parametric Kruskal–Wallis H-test was used to determine the differences of sulfur concentration in pigeon feathers' upper-shafts between habitats influenced by different anthropogenic usages. Then, the relationship between sulfur concentration and the sample weight in ravens was examined by using Pearson correlation test ($n = 19$). Since the pigeon data ($n = 50$) and the overall data set of both pigeon and raven failed to fulfill the assumption of parametric tests, the non-parametric Spearman correlation test was used.

To compare levels of trace element concentration between rural raven feather types and talons, samples of primaries upper shaft, wing coverts and talons were taken from eight rural ravens. One-way repeated measure ANOVA was used to compare the trace elements levels. Pairwise comparisons (LSD) were applied to determine between which of the samples significant differences occur. Since some trace elements (Cu and Pb) failed to fulfill the assumption of parametric tests, the non-parametric Friedman two-way analysis of variance by ranks test was used following by multiple comparisons between groups procedure [20]. Similar statistical analyses were used to compare the concentration of trace elements between raven feather parts (quill, middle feather, feather tip) from an industrial habitat. Eight raven primaries were divided into three sections: the quill, mid-shaft and upper-shaft (tip), and the concentrations of the 11 elements in each section were compared. In this dataset all trace elements fulfilled the assumption of parametric tests (for Al, Mn, Cu, Zn, Rb, Mo, Ba, Pb and Hg after logarithmic transformation) and there was no need to use non-parametric procedures.

To analyze the overall change in trace elements concentration in pigeon primaries (upper shaft) between locations of different anthropogenic uses (10 feathers from each location) multivariate analysis of variance (MANOVA) was used. A one-way ANOVA with Tukey HSD *post hoc* comparison was applied to determine which trace element contributes to the significant multivariate effect. Since the data was analyzed in a protected framework, it was not necessary to use the sequential Bonferroni adjustment [18] to decrease the chance of type I errors derived from family-wise error [19]. Since several trace elements (Mn, Zn and Hg) failed to fulfill the assumption of parametric tests, the non-parametric Kruskal–Wallis test one-way analysis of variance by ranks [20] was used. The non-parametric procedure for multiple comparisons with unequal sample sizes was applied to determine between which of the samples significant differences occur [21].

To compare levels of trace elements between ravens and pigeons in rural and industrial environments two-way MANOVA was used. Ten samples of primaries upper shaft were used of each species from each location (only nine samples from rural ravens). A univariate two-way ANOVA for each dependent variable was performed in order to determine which individual dependent variables contribute to the significant multivariate effect. Here too it was not necessary to use the sequential Bonferroni adjustment [17] since the data was analyzed in a protected framework.

3. Results

3.1. Sulfur content in feathers

Comparison of the coefficient of variation of all elements using all upper shafts samples (both ravens and pigeons) revealed that the variation of sulfur concentration is the lowest of all elements while the toxic elements mercury and lead show the largest variation (Fig. 1). Use of coefficients of variation (CV) makes it

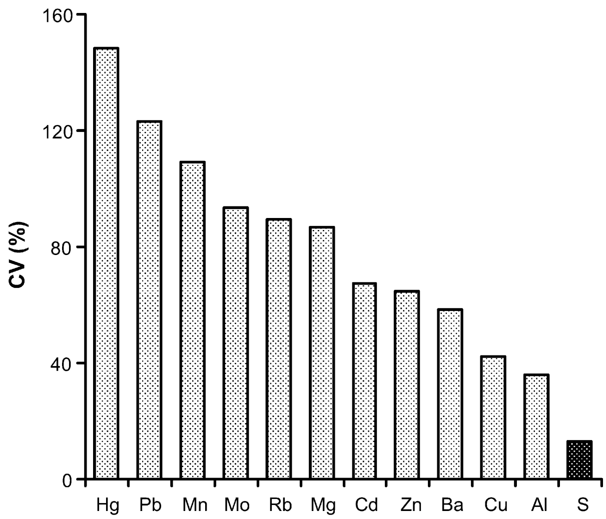

Fig. 1. Coefficient of variance (CV) of the concentration of 12 elements in the tip of pigeon and raven feathers ($n = 69$) from all habitats.

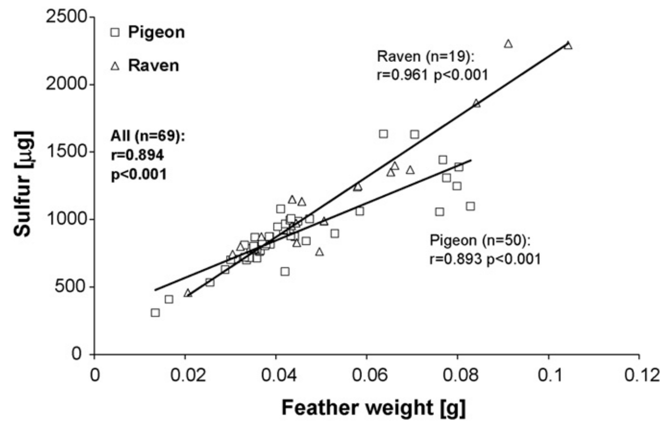

Fig. 2. The amount of sulfur in the feather samples as a function of the sample weight.

possible to compare the variation of all element concentrations, although their mean concentration differed by several orders of magnitude from each other.

The average content of sulfur in feathers was relatively high and consistent $2.14 \pm 0.28\%$ (raven = $2.2 \pm 0.26\%$; pigeon = $2.11 \pm 0.29\%$) of the feather weight. Thus, sulfur should be considered as a minor component in feathers, rather than a trace element. Comparison of sulfur concentration between different pigeon feather parts using one-way repeated measure ANOVA revealed a significant difference (d.f. = 2; $F = 12.55$; $P < 0.001$). Using pairwise comparison, the sulfur concentration in the quill ($1.92 \pm 0.07\%$) differed from the mid-shaft ($2.23 \pm 0.22\%$) and upper shaft ($2.25 \pm 0.13\%$) levels. For the proceeding analysis only one sample from each raven or pigeon (upper shaft) was used to avoid committing pseodoreplication. The non-parametric Mann–Whitney U-test revealed non-significant difference of the sulfur concentration in feathers between ravens and pigeons ($Z = -0.543$; $P = 0.587$). Similarly, non-significant difference was found when using the non-parametric Kruskal–Wallis H-test to compare the sulfur levels between pigeon feathers collected from different locations ($\chi^2 = 8.749$; d.f. = 4; $P = 0.068$). The non-parametric Spearman correlation test showed high and significant correlation between the sample weights and the sulfur concentration in the digested sample using 69 samples of upper shafts of both pigeons and ravens from the different habitats ($r = 0.894$, $P < 0.001$) (Fig. 2). Separate analysis for pigeons (Spearman correlation) and ravens (Pearson correlation) revealed a high and significant correlation (pigeon: $n = 50$; $r = 0.893$, $P < 0.001$; raven: $n = 19$; $r = 0.961$; $P < 0.001$).

3.2. Comparison of trace elements between raven feather types and talons

Comparison of toxic element concentration between raven primaries, wing coverts and talons revealed no differences in mercury levels and significant differences in lead and cadmium (Fig. 3a). For all toxic elements no difference was found between the two feather types and no other trend was revealed. Significant differences were found when comparing the levels of essential elements between the feathers and talons (Fig. 3b). With the exception of magnesium the concentration was always the lowest in the talons. For copper and aluminum no significant differences were found between the primaries and wing coverts. Concentration differences between the feather types occurred when comparing the magnesium, manganese and zinc levels where the concentration in the primaries was significantly higher than in the wing coverts. Comparison of the other elements revealed an inconsistent trend (Fig. 3c). No differences were found when comparing rubidium and barium levels, but for molybdenum the highest level was found in the primaries and the lowest in the talons.

3.3. Trace elements distribution along the feather shaft

Eight raven feathers were divided into three sections: the proximal quill, mid-shaft and distal upper-shaft (tip), and the concentrations of the 11 elements were measured in each section. Comparison of the distribution of the toxic elements (Hg, Pb and Cd) concentrations between the three sections of the feather showed that differences occurred in all three elements (Fig. 4a). For mercury and lead the highest concentrations were found in the upper shaft and the lowest in the quill while for cadmium relatively lower concentrations were found in the mid-shaft in comparison to the other two sections. Differences between essential element concentrations in feather sections were found in all elements, except copper in which no significant differences were found (Fig. 4b). When differences occurred, the highest concentrations were found in the distal tip. For zinc and manganese differences were observed also between the quill (lower) and the mid-shaft but no trend like this was found when comparing the magnesium and aluminum concentrations. The most consistent trend was found while comparing the distribution of the other elements along raven feathers (Fig. 4c). In all elements the concentration varied between the three sections: the lowest concentration at the quill and the highest concentration at the upper shaft.

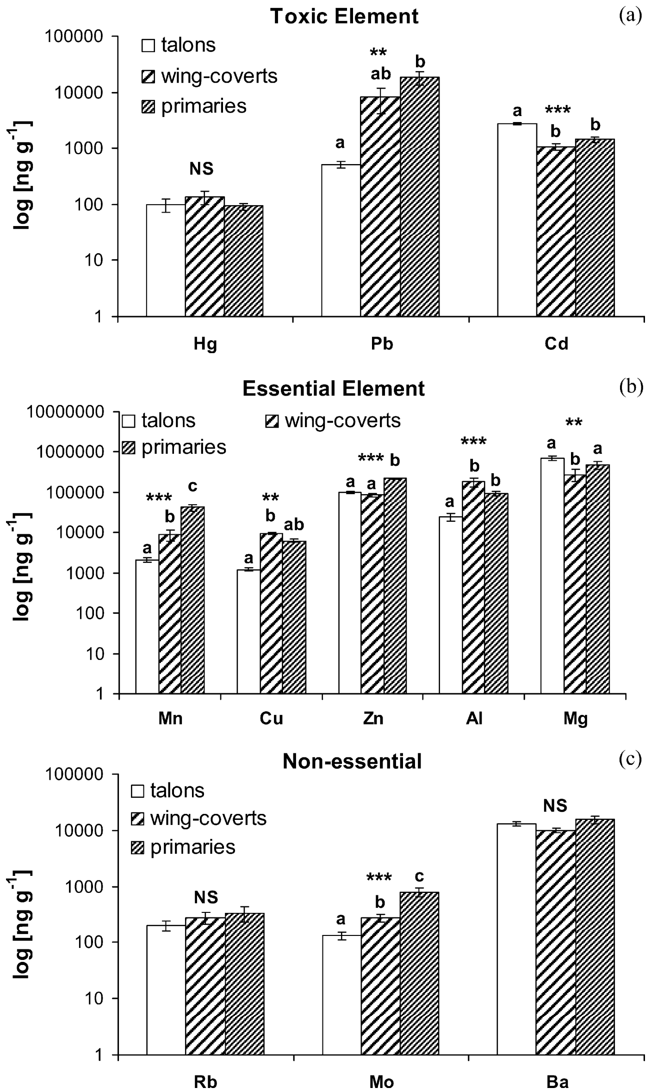

Fig. 3. The concentration of the 11 elements in raven primaries, covert feathers and talons. (a) Toxic elements; (b) essential elements; (c) non-essential elements. Asterisks denote the test significance level (*<0.05, **<0.01 and ***<0.001) and the letters a–c denote significant differences between primaries, wing coverts and talons (i.e., the same letter above different columns denote insignificant difference).

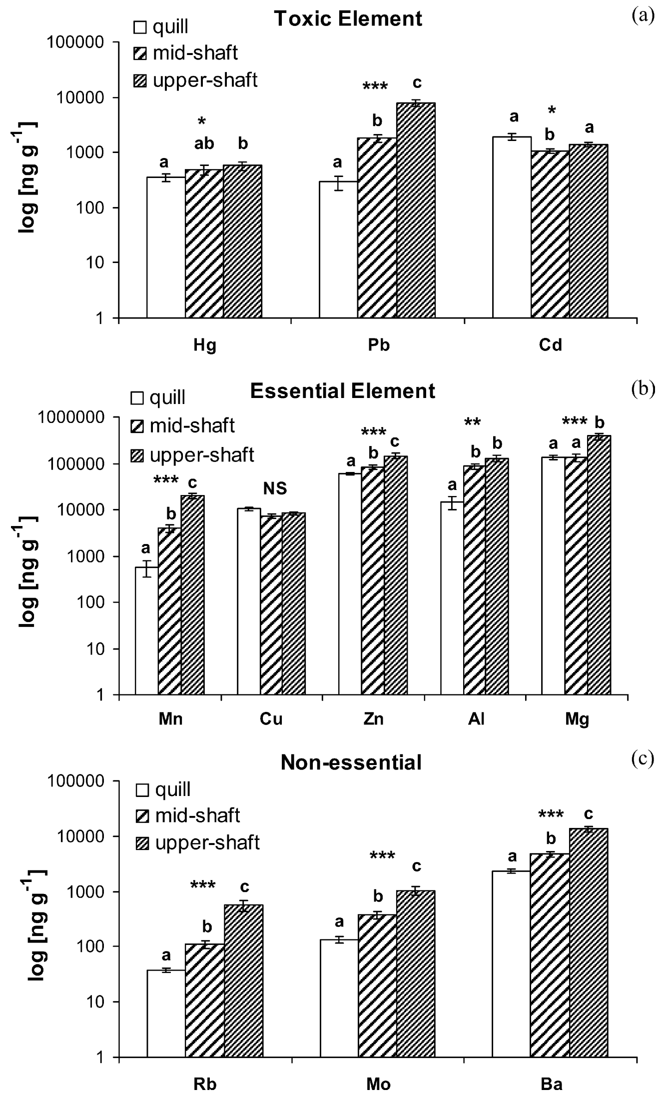

Fig. 4. The concentration of 11 elements between the three sections of the feather (proximal quill, mid-shaft and distal upper-shaft). (a) Toxic elements; (b) essential elements; (c) non-essential elements. Asterisks denote the test significance level (*<0.05, **<0.01 and ***<0.001) and the letters a–c denote significant differences between feather sections (i.e., the same letter above different columns denote insignificant difference).

3.4. Comparison of trace element levels between different habitat locations

Table 1 summarizes the average concentration and standard deviation of the measurements of the concentration of the 12 elements in the distal feather tips of pigeons ($n = 50$) and ravens ($n = 19$) at the different habitats. MANOVA testing for the overall change in trace elements concentration in pigeon feather tips between locations of different anthropogenic activities showed highly significant differences (d.f. = 44.144; Pillai's Trace = 2.488; $F = 5.385$; $P < 0.001$). A univariate F-test for each trace element with Tukey HSD *post hoc* comparison (or comparable non-parametric procedure described above) revealed that the concentration of all 11 trace elements vary significantly between different land uses ($P < 0.001$; Fig. 5a–c). The highest concentrations of the toxic elements mercury and lead were found in the industrial area and the lowest levels in the control and natural populations. Interestingly, no difference between locations was found for the cadmium levels except for low concentration in the natural population (Fig. 5a). Comparison of essential elements (Fig. 5b) showed that the concentration of all elements was lowest in the control population but otherwise no other noticeable trend was found. Relatively high concentrations of manganese and aluminum were found in feathers collected from the natural area. Comparing the concentration of the other elements (Fig. 5c) the only trend that was found was high concentration in the industrial population and relatively low concentration in the control population (except for molybdenum).

Table 1
The average concentration of ^{26}Mg, ^{27}Al, ^{34}S, ^{55}Mn, ^{63}Cu, ^{64}Zn, ^{85}Rb, ^{98}Mo, ^{114}Cd, ^{138}Ba, ^{202}Hg, ^{208}Pb in feathers of pigeons and ravens in μg g^{-1} (except sulfur that is in weight percent)

Average (μg g^{-1})	Pigeon					Raven	
	Control (n = 8)	Natural (n = 10)	Urban (n = 10)	Rural (n = 10)	Industrial (n = 10)	Rural (n = 9)	Industrial (n = 10)
Hg	0.03 ± 0.01	0.03 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.09 ± 0.02	0.09 ± 0.04	0.52 ± 0.16
Pb	0.29 ± 0.11	1.47 ± 0.75	6.99 ± 6.21	5.63 ± 4.38	10.3 ± 6.00	18.3 ± 14.5	15.4 ± 14
Cd	2.63 ± 1.07	0.91 ± 0.32	3.23 ± 2.9	1.84 ± 0.74	2.64 ± 0.4	1.44 ± 0.52	2.24 ± 0.97
Mn	0.42 ± 0.42	36.9 ± 15.2	9.7 ± 10.5	6.5 ± 2.5	5.44 ± 3.75	42.4 ± 23.8	21.8 ± 8.2
Cu	5.45 ± 1.26	5.72 ± 1.22	11.8 ± 4.6	9.77 ± 2.87	10.3 ± 2.8	6.31 ± 1.79	10.3 ± 3.2
Zn	28.7 ± 23.7	63 ± 27	131 ± 96	118 ± 29	146 ± 40	222 ± 30	171 ± 89
Al	68.9 ± 24.5	172 ± 44	134 ± 40	139 ± 30	154 ± 19	94 ± 32	153 ± 40
Mg	52 ± 31	394 ± 203	198 ± 151	178 ± 191	162 ± 73	483 ± 341	486 ± 153
Rb	0.06 ± 0.03	0.29 ± 0.27	0.18 ± 0.14	0.15 ± 0.06	0.32 ± 0.14	0.34 ± 0.32	0.54 ± 0.25
Mo	0.92 ± 0.94	0.17 ± 0.19	0.54 ± 0.42	0.63 ± 0.35	0.96 ± 0.99	0.80 ± 0.45	1.23 ± 0.73
Ba	4.84 ± 1.31	16.7 ± 8.1	11.31 ± 5.35	8.2 ± 2.7	11.96 ± 6.54	15.6 ± 7.36	18.3 ± 6.8
S%	2.21 ± 0.31	1.79 ± 0.38	2.12 ± 0.14	2.19 ± 0.07	2.25 ± 0.15	2.16 ± 0.31	2.23 ± 0.22

3.5. Comparison of trace elements between ravens and pigeons in two environments

Two-way MANOVA testing for the overall change in trace elements concentration between pigeon and raven upper shafts and between the two localities of different anthropogenic activities revealed highly significant interactions (location: d.f. = 11.24; Pillai's Trace = 0.918; F = 24.432; P < 0.001, species: d.f. = 11.24; Pillai's Trace = 0.923; F = 26.201; P < 0.001, interaction: d.f. = 11.24; Pillai's Trace = 0.798; F = 8.611; P < 0.001). Comparison of both rubidium and barium levels disclosed highly significant differences between the species and between rural and industrial populations (species: d.f. = 1.35; F = 5.487; P = 0.025; location: d.f. = 1.35; F = 17.028; P < 0.001, species: d.f. = 1.35; F = 13.988; P = 0.001; location: d.f. = 1.35; F = 4.985; P = 0.032, respectively). In all cases the concentration in raven feathers was higher than in pigeon feathers and the trace elements levels in the industrial environment was higher than in the rural environment (Table 1). No difference between species and localities was found when the molybdenum concentrations were compared. Comparison of magnesium and manganese levels showed significant differences between the species and no difference between localities (d.f. = 1.35; F = 33.033; P < 0.001, d.f. = 1.35; F = 46.551; P < 0.001, respectively). In both cases the concentration in ravens was higher than in pigeons (Table 1). An opposite pattern was found in a comparison of aluminum, cadmium and copper where no difference was found between species but a significant difference was found between rural and industrial areas (d.f. = 1.35; F = 13.234; P < 0.001, d.f. = 1.35; F = 8.183; P = 0.007, d.f. = 1.35; F = 8.847; P = 0.005, respectively). In all cases the trace elements concentration was higher in the industrial environment (Table 1). Significant interactions between the species and location were observed when comparing the zinc, mercury and lead concentrations (d.f. = 1.34; F = 6.522; P = 0.015, d.f. = 1.34; F = 30.369; P < 0.001, d.f. = 1.34; F = 4.338; P = 0.045, respectively). In all cases raven feathers had higher concentrations of these elements than pigeon feathers and feathers from the industrial environment contained higher levels than those collected from the rural areas, except for the levels of zinc and lead in ravens which were higher in rural environment (Table 1).

4. Discussion

The results presented above supported the main predictions and thus demonstrated that collection of common bird feathers from the ground followed by wet digestion and analysis by ICPMS might be an adequate method for coarse bio-monitoring of trace element levels in the environment. The concentration of 12 elements was determined, including toxic elements (Pb, Hg and Cd), essential elements (S, Mg, Al, Cu, Mn and Zn) and elements that cannot be considered as either toxic or essential like Rb, Ba and Mo (although molybdenum may play a vital role in some cases). It should be emphasized that the present study was concerned with the content inside the feather, after removal of external contamination.

The content of sulfur in feathers is relatively high, thus it should considered as a minor component in feathers. This is mainly due to the presence of the amino acid cystine that is a key component of keratin (a protein that is of special importance in hair, wool, horns, nails, etc.). The average concentration of sulfur in all feather parts from all habitats for both pigeons and ravens was found to be 2.14 ± 0.28% and the variations in its content were much smaller than of any of the other 11 elements determined in this study, as shown clearly in Figs. 1 and 2. A meticulous statistical analysis showed that there were small but significant differences between the sulfur content in the quill and the vane, but not between the feathers of ravens and pigeons or between pigeon feathers collected from five different habitats. The average content of sulfur in human hair and nails has been reported as 4.77 ± 0.41% and 3.3 ± 0.54%, respectively [22] and sulfur has been used as an internal standard for laser ablation studies of human hair [23]. The results of the present work demonstrated that sulfur can be suitable to serve as an internal standard for measurements of trace elements in feathers, in a similar fashion to its use in studies of trace elements in human hair. This would of particular interest for studies involving laser

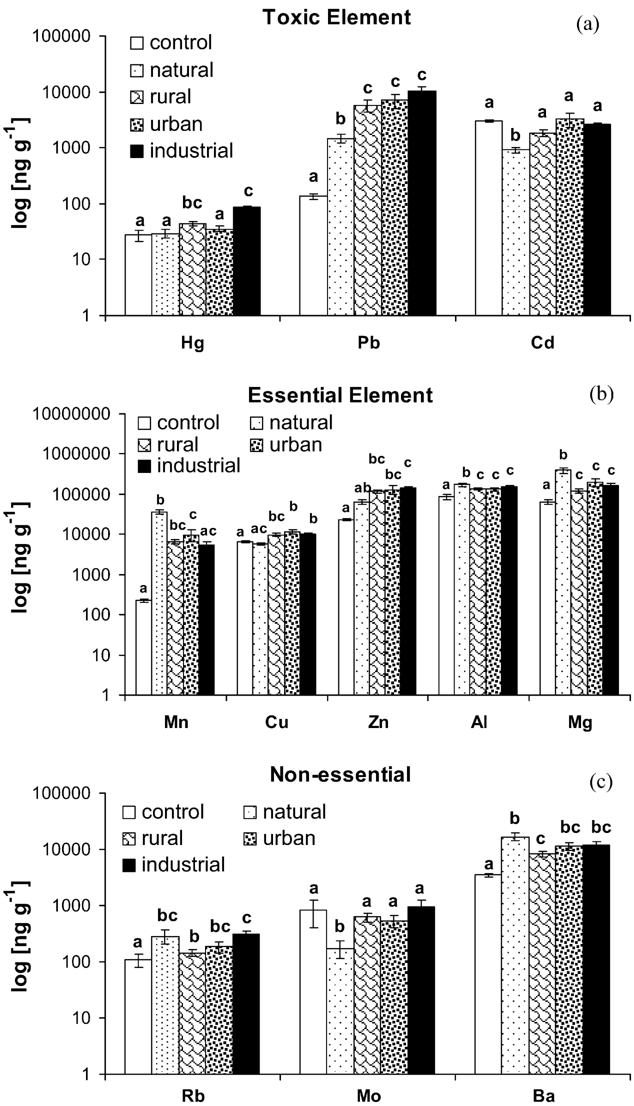

Fig. 5. The concentration of the 11 elements in pigeon feathers from controlled, rural, natural, urban and industrial areas. (a) Toxic elements; (b) essential elements; (c) non-essential elements. Asterisks denote the test significance level (*<0.05, **<0.01 and ***<0.001) and the letters a–c denote significant differences between locations (i.e., the same letter above different columns denote insignificant difference).

ablation-ICPMS studies of feathers where an internal standard is needed to correct for variations in the amount of ablated material [9,23–24].

When comparing the concentration of trace elements between raven feather types and talons no differences were found for most elements between the feather types. In the few cases where differences did occur the trace element concentration was higher in the primaries feathers. These findings make it possible to recommend using the primaries to examine concentration of trace elements in the environment. Interestingly, here too cadmium presented a different trend than most other elements—talons exhibited higher levels than feathers.

Comparing the general trend of trace element distribution along the feather supported previous studies that revealed increased levels of elements while moving from the proximal quill to the distal upper shaft. The distribution of most elements along the feather shaft shows that for manganese, zinc, rubidium, molybdenum, barium and lead there is a pronounced increase in concentration from the quill, through the bottom and middle of the shaft toward the tip of the feather. A similar trend for mercury and lead was reported by Burger and Gochfeld who also noted that no significant difference was found for selenium and cadmium [25]. However, unlike other studies that considered external contamination as a possible explanation for this trend, the rinsing protocol that was used in the current study and was developed according to the procedure used for samples of human hair and nails [15–16] is considered to minimize the possibility of external contamination. An alternative explanation that was proposed is that during feather growth the blood circulation in the feather helps deposit toxic and non-essential elements and surplus of essential elements at the distal edge of the feather, so that once feather growth stops and blood no longer circulates through the feather, the feathers serve as a convenient depository for unwanted elements. However, the fact that the most consistent trend of increasing concentration toward the feather upper-shaft was found in the non-essential elements and not in toxic elements does not support this explanation. Similarly, although toxic, cadmium concentration did not differ between the quill and the upper-shafts. Another possible explanation that was proposed previously is that differences in feather structure allow different binding capabilities [6]. Such an explanation may account for the different trends found between different elements in the current study.

Trace element concentrations reported herein (Table 1) are comparable to those obtained in other studies, although a detailed comparison is not possible due to the fact that in those studies either a different suite of elements was examined, different bird species were studied or birds from very different habitats were investigated. For example, the average content of aluminum in all the feather samples was $102 \pm 70\ \mu g\ g^{-1}$ and this can be compared with the values of 39–49 $\mu g\ g^{-1}$ reported by Dauwe [6] for birds of prey or 81–315 $\mu g\ g^{-1}$ for great tits and blue tits in polluted and reference sites as reported by Eens [10]. The average value obtained for manganese in the present study ($10.8 \pm 16.1\ \mu g\ g^{-1}$) is quite similar to 11.1–15.5 $\mu g\ g^{-1}$ reported by Dauwe [6] for birds of prey or 8.77 $\mu g\ g^{-1}$ reported by Nam for cormorants [3]. Industrial use of fossil fuels and minerals, the use of insecticides and inorganic fertilizers in agriculture and accumulation of toxic wastes in urban areas all contribute to increase the levels of trace elements above the concentration naturally present in the environment. As a result, trace elements accumulation in the environment differs according to the anthropogenic land uses in each area. The lowest concentration of most studied elements was found in pigeon feathers from the control population. Higher levels in all other populations indicate that the methodology adopted in the present work is appropriate for revealing the accumulation of trace element in the ecosystem. Similarly, the general trend that was observed in the current study agrees with the predicted trend—increasing trace elements' concentration as the land use practices become industrialized. Especially, relatively high concentrations of the toxic elements mercury and lead in industrial areas and their lowest concentration in pigeon feathers from the natural environment indicate that toxic element pollution exists in industrial sites in the Negev desert. Elevated levels of most studied elements, including cadmium, that were found in raven feathers from the industrial habitat further support this claim. Another interesting insight is the similarity in the levels of most studied elements between rural and urban environments. Daily movements of pigeon flocks from urban roosting areas to the foraging grounds in rural areas may obscure possible differences between the two habitats. Unpredicted high concentration of aluminum, magnesium and manganese in pigeon feathers from natural environment raise the question: what are the natural or artificial sources from which these elements penetrate the ecosystem? More detailed studies using bioindicators of finer resolution are needed to expose these sources. Trace elements accumulate in tissues of plants and animals, thus species positioned higher in the food web are expected to comprise higher levels of trace elements. As expected, higher concentrations were found in raven feathers in comparison to pigeon feathers for most studied elements (manganese, magnesium, barium, rubidium, mercury, lead and zinc).

5. Conclusions

The present study demonstrated that feathers of common resident birds collected from the ground can be used for coarse monitoring of the level of trace elements in the environment. When using feathers to monitor trace element concentrations it is recommended to sample the primaries upper-shaft. The use of wet digestion and ICPMS provides a powerful analytical technique for determination of the concentration of trace elements in feathers. Sulfur was found to be an adequate element to serve as an internal standard and wet digestion followed by ICPMS analysis gave results that were in agreement with those obtained by other procedures or analytical techniques. First indications for environmental pollution in one of the Negev desert industrial areas were found and indications of unknown sources of aluminum, magnesium and manganese were found in the Negev mountains nature reserve. Future monitoring plans can use the methodology tested herein to reveal trace elements' pollution in a coarse scale and to examine if anthropogenic contamination can be the reason for endangered bird populations decline.

Acknowledgment

We would like to thank the Israel Nature and Parks Authority employees: Yoni Sharir; Nimrod Ben-Aharon; Raviv Shapira, and especially Michael Blecher for collecting the feathers.

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