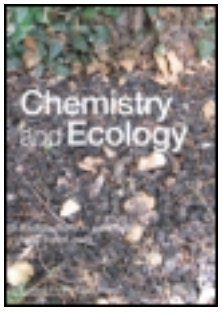

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Miroslav A. Demajo [a], Jelena Cvetićanin [a], Milovan Stoiljković [a], Djordje Trpkov [a], Velibor Andrić [a], Antonije Onjia [a] & Olivera Nešković [a]

[a] Institute of Nuclear Sciences 'Vinča', Belgrade, Serbia

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Miroslav A. Demajo, Jelena Cvetićanin, Milovan Stoiljković, Djordje Trpkov, Velibor Andrić, Antonije Onjia and Olivera Nešković*

Institute of Nuclear Sciences 'Vinča', Belgrade, Serbia

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In this study, we analysed pellets from long-eared owls (*Asio otus*) collected from four localities in Belgrade (Serbia). The pellets contained the remains of prey, namely voles (*Arvicola terrestris*) and field mice (*Apodemus agrarius*). The concentrations of 14 elements (Ca, P, Mg, Na, K, Fe, Zn, Sr, Ba, Mn, Ti, Cu, Si, B) were evaluated in whole pellets and in samples containing only bone tissue, which were dissected from the whole pellet. The increased levels of certain elements, including Mn, Zn, Ba, Cu and radioactive ^{40}K, indicate contamination of the soil by various sources, such as industrial plants and agricultural practices. From the results presented in this article, we suggest that the analysis of owl pellets may indicate the quality of the local environment.

Keywords: owl; *Asio otus*; pellets; element content; radioactivity

1. Introduction

Harmful wastes emitted from industrial plants and heavy traffic combined with the various pesticides and fertilisers used in agriculture are regular sources of soil contamination. Controlling the accumulation of toxic substances in ecosystems is of great importance in trying to decrease global atmospheric pollution. It has been shown that pollutants in a specific region accumulate in living organisms, and the advantages of environmental biological monitoring are clear [1]. Biological monitoring is even more advantageous when specimens for analysis are obtained using noninvasive methods. This is the case with owl pellets, which are readily available and contain rodent remains, bones and hair, which can serve as indicators of environmental pollution [2].

Previously, it has been demonstrated that by analysing the tissues of various small mammals, especially rodents, it is possible to evaluate the quality of the local environment. In particular, environmental radioactivity and heavy metal contamination can be monitored using this method [3–7]. In these studies, rodenticide residues in rodents were studied using barn owl (*Tyto alba*) pellets [8]. In addition, organochlorine pollutants in the eggs of the little owl (*Athene noctua*) [9]

*Corresponding author. Email: oliveran@vinca.rs

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and the accumulation of heavy metals in the bones and faeces of a number of bird species have also been analysed [10].

In this study, we describe methods of preparing owl pellets for monitoring soil conditions in specific localities by determining the pellet element content and the environmental radioactivity.

2. Materials and methods

2.1. *Sample collection*

The long-eared owl (*Asio otus*) is widely distributed in Europe [11]. During the winter months, the birds roost in trees, often in urban areas, in numbers ranging from several individuals to flocks with over 100 birds per tree. Mounds of owl pellets can easily be found under these roosting trees.

On 17 January 2010, pellets were collected from four locations around the city of Belgrade (Serbia). The geographical coordinates of these locations were as follows (coordinates from Google Earth satellite images): 'Lešće' Cemetery (44°48′28.44″N; 20°32′46.63″E), 'Bežanijska kosa' Cemetery (44°48′55.80″N; 20°22′04.59″E), 'Novo groblje' Cemetery (44°48′31.86″N; 20°29′28.73″E) and the 'Vinča' Institute (44°45′16.37″N; 20°36′15.98″E).

Some of the pellets collected from each locality were processed for analysis, whereas others were first dissected to separate the bones from the hair, so that the bone tissue only could be analysed. During this dissection process, it became obvious that only rodent remains were present [12]. The skeletal remains belonged to ground voles (*Arvicola terrestris*) and field mice (*Apodemus agrarius*). These rodent species are the staple prey of the long-eared owl during the winter months.

2.2. *Mineralisation of samples*

Ten pellets from each locality were pooled and further processed for analysis. For the bone samples, 10 pellets from each locality were dissected, and their bone content was pooled. The collected pellets were dried at 100 °C for 2 h. For the analysis, 2.5 g of material from the whole pellet and 1 g of bone samples were used (measurements had a precision of 10 mg) and transferred to glass beakers. A 1:1 mixture of H_2O and 10 M HNO_3 (40 mL) was added and the solution was left overnight at room temperature. The next day, the covered beakers were heated slowly. A significant amount of brown fumes developed from the beakers containing whole pellets, followed by a moderate amount of foam that subsequently disappeared. The solutions containing the samples were then evaporated to dryness and cooled. Next, 15 mL of 10 M HNO_3 solution was added to each sample, and the solutions were then evaporated to one third of their original volume. After cooling, 3 mL of 7 M $HClO_4$ was added to each sample. The solutions were heated slowly until white fumes appeared, and the bone samples were completely mineralised. Whole-pellet samples showed the presence of a heavy precipitate, and the solution itself was a light yellow colour. Samples were collected via filtration and transferred to 50-mL Erlenmeyers and HNO_3 was added to reach a final concentration of 10%. Each sample was measured five times, and the results are presented as the mean ± SD.

2.3. *Sample preparation for radioactivity measurement*

Before other analyses were applied, 50-mL solutions of the mineralised samples were used for gamma spectrometry measurement.

2.4. *Preparation of the samples for mass spectrometry measurement*

An aliquot of each sample solution containing the internal standard was combined in a 1:1 ratio with the CHCA (α-cyano-4-hydroxycinnamic acid) matrix. The solutions were mixed thoroughly. Aliquots (0.5 μL) of the mixtures were spotted on a sample plate and allowed to dry in air. Mass analysis was carried out in positive ion reflector mode using a 200 Hz N_2 pulsed laser operating at 327 nm. Five spectra were collected per spot (between 170 and 300 Da) at each of 10 randomly selected positions.

2.5. *Instrumentation used for measurement of element content and radioactivity*

An inductively coupled plasma optical emission spectrometer (ICP-OES) was used to evaluate the levels of Ca, P, Mg, Fe, Zn, Sr, Ba, Mn, Ti, Cu, Si and B in the samples. A Spectroflame ICP-OES spectrometer (Spectro Analytical Instruments, Germany) operating at 27.12 MHz and 2.5 kW was used. The instrument was equipped with four polychromators, enabling simultaneous determination of 30 elements, and then used a single monochromator for sequential measurements. For evaluation of Na and K levels, U-shaped direct current argon arc plasma emission spectrometry (DCP-OES) was employed [13]. In this spectrometry measurement, argon arc plasma is spatially stabilised by combining gas vortex and wall stabilisation. Analytes were introduced into the plasma by spraying solutions using a Mainhard pneumatic nebuliser associated with a Scot-type nebuliser chamber.

The calibration standards were prepared in appropriate concentrations from elemental stock solutions. Matrix-matched calibration standards were provided.

A Voyager de Pro (AB Applied Biosystems, UK) matrix-assisted laser desorption/ionisation time-of-flight mass spectrometer (MALDI-TOF MS) was used to detect the possible presence of Cs, Sr, U and Am in the samples. The detection limit for ionic species is in the femtomolar range.

The ^{40}K isotope levels were measured using low-background gamma spectrometry and an ORTEC high-purity germanium (HPGe) detector of 38.60% relative efficiency. For the calibration, a standard solution of KCl (Merck, p.a.) was used in a concentration of 5 g per 50 mL. Samples were measured in cylindrical vessels 7 cm in diameter. The same geometry was used for measuring the blank sample (deionised water) and standard solution.

Alpha and beta radioactivity measurements were performed on a Protean MPC9400 low background gas proportional counter. The counter was calibrated with ^{241}Am for gross alpha determination and with ^{90}Sr for gross beta determination.

3. Results and discussion

As illustrated in Figures 1 and 2, the element content patterns that were measured in skeletal tissue and whole pellets, which included undigested hair, were similar. The basic constitutive elements of mammalian bone tissue, Ca, P, Mg, Na and K, were expected to be found in the greatest amounts [14]. According to the quantity ratio of Ca, P, Mg and Sr in bone samples, the whole pellets contained $\sim$70% bone tissue.

From Table 1, it can be seen that the highest variation in the samples of bone tissue between the localities was found for the Mn and Si content. Whole-pellet samples show distinct locality variations in Mn, Ti and B content.

To understand the variability in the element content detected in the samples, it is necessary to refer to the results of geological surveys of the areas from which the pellet samples were obtained [15]. All the localities from which the pellet samples were obtained are geologically composed

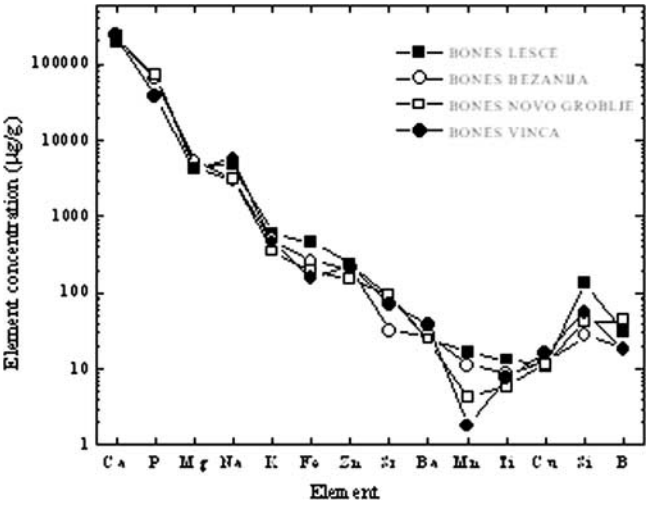

Figure 1. Element concentrations in rodent bone samples separated from whole owl pellets.

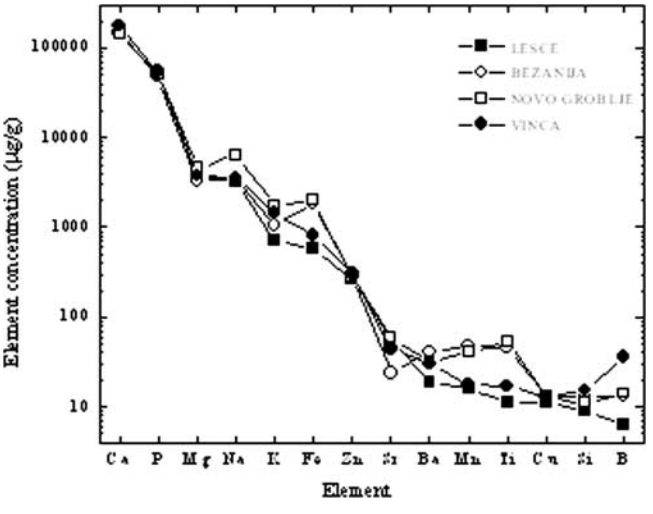

Figure 2. Element concentrations in whole owl pellet samples.

primarily of a mixture of sand, clay and marl. The specified localities are completely or partially surrounded by agricultural land, with only the Novo Groblje Cemetery being in the city centre. However, owls from this locality are not more than a 2-km flight distance away from agricultural land and feeding grounds.

From this brief description of the local geological characteristics, it is understandable that a high bioaccumulation of Si is detected in the bone tissue. It is of interest that the highest Si content was visible in samples from the Lešće Cemetery and the Vinča Institute, which are located near natural loess deposits on the banks of the Danube River. Silica is not considered an essential element, although it does seem to play a role in the physiology of mammals. Silica can enter the body through the ingestion of drinking water that contains silica from the surrounding ground substrate. This is especially true in plants, which contain adherent soil particles that rodents, predominantly herbivores, then freely ingest [16]. The presence of silica also explains the detection of Ti in our

Table 1. Concentrations of detected elements measured using ICP-OES and DCP-OES

Sample location	Ca ($mg \cdot g^{-1}$)	P ($mg \cdot g^{-1}$)	Mg ($mg \cdot g^{-1}$)	Na ($mg \cdot g^{-1}$)	K ($mg \cdot g^{-1}$)	Fe ($mg \cdot g^{-1}$)	Zn ($\mu g \cdot g^{-1}$)	Sr ($\mu g \cdot g^{-1}$)	Ba ($\mu g \cdot g^{-1}$)	Mn ($\mu g \cdot g^{-1}$)	Ti ($\mu g \cdot g^{-1}$)	Cu ($\mu g \cdot g^{-1}$)	Si ($\mu g \cdot g^{-1}$)	B ($\mu g \cdot g^{-1}$)
						Bone only								
L	200 ± 10	69.0 ± 3.0	4.6 ± 0.2	4.7 ± 0.2	0.60 ± 0.01	0.47 ± 0.04	240 ± 20	85 ± 2	25 ± 1	16 ± 2	13.4 ± 0.5	10.8 ± 0.5	136 ± 4	31 ± 4
B	240 ± 10	64.0 ± 4.0	5.4 ± 0.1	3.0 ± 0.1	0.50 ± 0.02	0.26 ± 0.01	210 ± 10	31 ± 1	28 ± 1	11 ± 1	8.75 ± 0.04	12.5 ± 0.6	28 ± 2	19 ± 2
NG	230 ± 10	71.0 ± 4.0	4.2 ± 0.1	3.2 ± 0.3	0.35 ± 0.01	0.19 ± 0.01	152 ± 7	93 ± 1	25 ± 1	4.3 ± 0.3	5.9 ± 0.1	11.6 ± 0.7	41 ± 3	43 ± 3
V	240 ± 9	80.0 ± 2.0	4.3 ± 0.2	5.8 ± 0.4	0.46 ± 0.04	0.16 ± 0.02	220 ± 30	71 ± 2	38.4 ± 0.9	1.8 ± 0.4	7.7 ± 0.2	16.1 ± 0.9	55 ± 2	18 ± 4
Mean $\pm$ SD	228 ± 19	71 ± 7	4.6 ± 0.5	4 ± 1	0.5 ± 0.1	0.3 ± 0.1	206 ± 38	70 ± 28	29 ± 6	8 ± 6	9 ± 3	13 ± 2	65 ± 47	28 ± 12
						Whole pellet								
L	140 ± 3	51.0 ± 2.0	3.75 ± 0.06	3.2 ± 0.1	0.70 ± 0.06	0.57 ± 0.02	270 ± 30	54.6 ± 0.3	19 ± 2	16 ± 3	11.4 ± 0.3	11.4 ± 0.3	8.9 ± 0.6	6.3 ± 0.6
B	148 ± 4	48.0 ± 1.0	3.3 ± 0.2	3.5 ± 0.2	1.04 ± 0.09	1.83 ± 0.04	280 ± 40	24 ± 2	41 ± 7	48 ± 9	46.0 ± 2.0	13.0 ± 0.4	13 ± 2	13 ± 2
NG	145 ± 4	50.0 ± 0.3	4.6 ± 0.2	6.4 ± 0.1	1.8 ± 0.2	1.99 ± 0.07	280 ± 40	58 ± 1	32 ± 4	41 ± 5	53.0 ± 2.0	13.1 ± 0.3	11.0 ± 7	14 ± 2
V	176 ± 8	55.0 ± 3.0	3.7 ± 0.2	3.6 ± 0.3	1.4 ± 0.2	0.82 ± 0.05	300 ± 30	44 ± 3	30 ± 3	18 ± 2	17.0 ± 0.3	13.2 ± 0.6	15 ± 2	36 ± 3
Mean $\pm$ SD	152 ± 16	51 ± 3	3.8 ± 0.5	4 ± 1	1.2 ± 0.5	1.3 ± 0.7	282 ± 12	45 ± 15	31 ± 9	31 ± 16	32 ± 21	12.8 ± 0.8	12 ± 3	17 ± 13

Notes: E, element with unit of measurement, values given as mean $\pm$ SD. L, Lešće; B, Bežanijska kosa; NG, Novo Groblje; V, Vinča Institute.

samples, as it has been shown that increased silica intake stimulates Ti bioaccumulation, especially in the form of TiO_2 [17].

Certain differences in the content of specific elements in the two sample types were to be expected, as different tissues accumulate certain elements at different rates and have different affinities for specific elements [18]. In our study, we had samples of pure bone tissue and samples with a mixture of bone and hair. If average values from all localities for each element in the two sample types (Table 1) are expressed as percentages, higher levels of Ca, P and Mg (48.9, 18.7 and 19.8%, respectively) are expected in bone tissue samples than the whole pellet, because these elements are the main inorganic constituents of bone. The following elements also show relatively higher concentrations in the bone samples than in whole pellet samples: Na, 0.24%; Sr, 55.56%; Cu, 0.31%; B, 60.22% and Si as an extreme, has levels 5.4 times higher.

The higher than expected accumulation of Zn in the bones and whole pellets can be explained by possible residual Zn in soil from surrounding agricultural land, possibly originating from the rodenticide zinc phosphide [14,19]. Although its use is now severely restricted by law, illegal use is still practised. The deaths of barn owls (*Tyto alba*) and buzzards (*Buteo buteo*) due to poisoning by this chemical have been recently recorded in Serbia [20]. According to the quantity ratio of Zn in bone tissue and whole pellets, it is obvious that Zn accumulates $\sim 30\%$ more in the hair fraction of the pellet.

The elements Ti, Sr, Ba and Cu that were found in our samples are readily deposited as part of petrochemical waste and then are accumulated by rodents such as the cotton rat (*Sigmodon hispidus*) [21]. Pollution of agricultural land, especially around urban areas, is a regular occurence, and the presence of large petrol refineries in the town of Pančevo, only a 12 km flight from Belgrade, is also a regular source of pollution. The presence of these elements in our samples is thus expected and may indicate heavy soil contamination. The detection of Cu in our samples can also be explained by the fact that orchards and sporadic vineyards, in which copper-based fungicides are used, are on the agricultural land around the localities where the samples were obtained. Thus, Cu may be present in topsoil in amounts far above its natural background concentrations [22]. Moreover, the quantities of both Cu and Ba are equal in both sample types.

The detection of Ba in both of the sample types is notable, because this element is not usually found in concentrations that are toxic to rodents. It is, however, readily accumulated by plants, especially legumes, and thus ingested by small mammals. Barium may be found in various metal industries [23].

The presence of Mn in our samples is in agreement with the findings of Vukojević et al. [24] who detected increased levels of this element deposited on four moss species in the vicinity of Belgrade. Our pellet samples were also analysed for the presence of Pb (by ICP-OES), but this element was under the detection limit of the applied analytical method. Currently, there is no explanation for this result, as one would expect an increased amount of this element in an urban environment.

This tudy demonstrates the general distribution of specific elements in owl pellet samples and shows that element concentrations differ between localities. There are data from literature regarding the detection of certain elements, such as F and the presence of radioactivity, in owl pellets [25,26]. In this study, we succeeded in measuring a wider range of elements.

Although some levels of radioactive elements, such as ^{137}Cs and ^{40}K, have been reported in skulls of rodents and insectivorous animals found in owl pellets from Poland [25], we detected only the presence of ^{40}K. Measured specific activities of ^{40}K were in the range 19.4–55.2 $mBq \cdot g^{-1}$, which corresponds to the K content found in samples (0.61–1.74 $mg \cdot g^{-1}$). This is in accordance with our results derived from ICP analysis (Table 1). Because the sample quantity was very small, ^{40}K activity was very low and the uncertainty of the measurement was thus $\sim 30\%$, although measuring times varied from 64,000 to 330,000 s. The estimated MDA for this geometry is $\sim 3.25\ mBq \cdot g^{-1}$. Average specific radioactivities of ^{40}K in soils were $\sim 700\ Bq \cdot kg^{-1}$,

according to monitoring studies for the Belgrade area. According to Grdović et al. [27], agricultural soil around the city of Belgrade does contain ^{40}K. Our MALDI-TOF MS showed only molecules of collagen and hydroxyapatite, the main bone-building materials. Molecular or atomic ion species, which might contain Cs, Sr, U and Am as end products of radioactive disintegration, were not detected. It is well known that one of the important anthropogenic sources of environmental pollution is phosphorus-based fertilisers [28], which may explain the differences in the content of ^{40}K in our analysed owl pellets.

Becker states that birds, especially birds of prey, have an important role as bioindicators [29]. This author also notes that birds are still not used as effectively as possible for monitoring the environment, an area to which our research is a contribution.

4. Conclusions

Rodent remains in owl pellets may serve as good indicators for monitoring the quality of local soil and environments in general. The pattern of element distribution in the owl pellets reflects the geological and soil characteristics of the studied localities. With this sampling method, it is not necessary to trap and kill rodents as sample sources.

To achieve a clearer insight into environmental conditions, especially in the soil, it may be advisable, according to our experience, to analyse owl pellets during the winter months when they are likely to contain fewer remains of other prey, such as birds and insects. Rodents are more resident and may better reflect the conditions of specific local environments.

It is necessary to emphasise that, as in many other countries, all 10 Serbian owl species are protected by law as natural rarities and useful species that effectively control rodent populations and help reduce the need to apply environmentally harmful rodenticides. Using owl pellets as a sample source is a harmless and noninvasive sampling method that is safe for the owls.

Acknowledgements

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