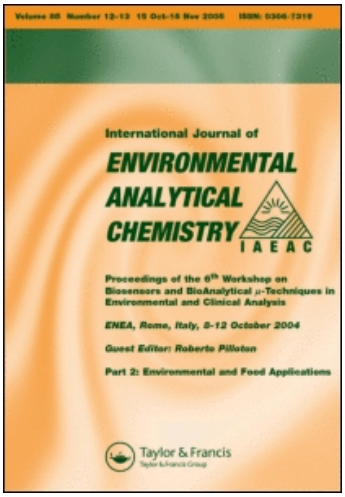

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DETERMINATION OF TRACE METALS IN WOODLICE AND THEIR SKINS WITH PARTICULAR EMPHASIS ON QUALITY CONTROL

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Al, Fe, Mn and Zn were determined in two different species of woodlouse: *Porcellio scaber* and *Porcellio dilatatus*. Both species were cultivated under standardized conditions in a climatic chamber. Moreover, skins of the cultivated animals were collected and analyzed separately to examine a possible way of decontamination by moulting. To obtain enough sample material for each species, 15 animals of the same age and size were pooled. For skin analysis, 10–12 skins were collected and pooled. The animals and their skins were dried, ground and digested in pure concentrated nitric acid using multiwave-assisted high-pressure digestion and, finally, analyzed by ICP-OES. Special emphasis was given to quality control: the reference materials Dorm-2, Dogfish Muscle (*Squalus acanthias*) and SRM 1577b Bovine Liver, were used to evaluate the whole analytical process including sample digestion. These reference materials of animal origin were selected to match the matrix of the samples as closely as possible. While concentrations of Fe, Mn and Zn were traced to both reference materials, the concentration of Al was checked by Dorm-2 only. Analyses of the elements in the reference materials were carried out using four different wavelengths for each element simultaneously, aiming at the determination of the best suited wavelength for each element. Analyses of woodlouse samples and their skins were finally carried out using the wavelengths with the highest sensitivities after absence of spectral interferences had been demonstrated.

Keywords: Quality control; Standard reference materials; Trace metals; Woodlice

INTRODUCTION

The woodlice *Porcellio scaber* (Latreille, 1804) and *Porcellio dilatatus* (Brandt, 1833) are considered to be good bioindicators of metal contamination of the terrestrial environment [1] as well as of metal accumulation in saprophagous food chains [2]. The selected species are widespread throughout Central Europe and the United States in both rural and urban areas. They are relatively large, conspicous and easily collected, and show a high tolerance for heavy metals by accumulating them in vesicles in the hepatopancreas [3]. The animals inhabit the upper layer of the soil and surface leaf litter, which is an important sink for metal contaminants [4]. Dead organic matter, as well as cryptogamous plants such as lichens and mosses accumulate high amounts of heavy metals

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due to the high stability of the chemical complexes formed between heavy-metal ions and the negatively charged organic groups of the plants as well as ion-exchange facilities which allow the effective adsorption of metal ions to occur. However, woodlice are reported to be able to excrete at least parts of the accumulated metal ions [5]. We analyzed skins of the animals for Al, Fe, Mn and Zn to examine whether moulting was a means of excretion of metals by woodlice.

The main goal of our study, however, was to demonstrate both the accuracy and reliability of the analytical procedure for trace metal analysis in woodlice. For this purpose, two reference materials of animal origin, Dorm-2, Dogfish Muscle (*Squalus acanthias*) and SRM 1577b Bovine Liver, were applied to match the matrix of the samples as closely as possible, which is a prerequisite for accurate results. Many data on metal concentration in woodlice, however, lack careful quality control and are therefore inappropriate for risk assessment.

Moreover, there is still a need for additional data on the metal content of 'uncontaminated' woodlice, i.e. animals that have not been collected from contaminated sites. Consequently, our data should serve as a basis to assess metal contamination reliably. The animals we analyzed were cultivated under standardized conditions to avoid metal contamination.

In a later step, the method was used to analyze numerous batches of woodlice collected from a heavily contaminated former industrial site near Jena (Thuringia) to evaluate risk assessment. These data, however, are not the subject of this article.

EXPERIMENTAL

Sample Preparation

Total Animals

Woodlice of the species *P. scaber* and *P. dilatatus* were permanently cultivated under standardized conditions in a climatic chamber at a constant temperature of 22°C and a humidity of >90%. The animals were fed exclusively with fresh carrots and lettuce purchased from the same supplier. However, no specific provision control was made. No water was additionally provided to the animals. To avoid a possible trophic enrichment of minerals by cannibalism, the density of the cultivated populations was regularly checked. After running the culture for one year, 15 animals per species of the same age and size, corresponding to a dry weight of 30–40 mg, were picked out manually and killed by cooling to −18°C. The pooled samples were then freeze-dried and eventually ground by a planetary ball mill (Retsch, Haan, Germany) cooled with liquid nitrogen. Aliquots of about 100 mg of ground animal material were then subjected to digestion and analysis. For further details on sample preparation, see Rothe and Gleixner [6].

Skins of Animals

Skins of *P. scaber* and *P. dilatatus* were collected separately and manually after moulting about every 2–3 weeks. Pooled samples from 6 months of skin collection were dried, stored and ground as described earlier but without additional cooling by liquid nitrogen. Subsamples of 50 mg, equivalent to 10–12 skins of adult woodlice of the same age and size, were used for digestion and analysis.

TABLE I ICP instrumental operating parameters (upper part) as well as wavelengths used for analyses of reference materials (lower part)

Parameter	*Set-up*
RF power	1300 W
Plasma gas	15 L/min
Auxiliary gas	0.5 L/min
Nebulizer gas	0.8 L/min
Pump rate	1.3 mL/min
Nebulizer	Cross-flow
Spray chamber	Scott-type, Ryton
Plasma view	Axial
Viewing distance	15 mm
Element	*Wavelength* (nm)
Al	237.313, 308.215, 309.271, 396.152
Fe	234.349, 238.204, 239.562, 259.940
Mn	257.610, 259.372, 260.586, 293.305
Zn	202.548, 206.200, 213.857, 334.501

Sample Digestion

About 100 mg of standard reference material or pooled animal material, respectively, was used for microwave-assisted high-pressure digestion (Multiwave, Anton Paar, Graz, Austria) after the addition of 3 mL of 65% suprapur HNO_3 (Merck, Darmstadt, Germany). A six-position rotor with 50-mL reaction vessels made of tetrafluor-modified polytetrafluoroethylene was used. The maximum reaction temperature was 230°C with maximum pressures of 25–30 bar. Overall, the digestion consisted of three steps: (1) increase in microwave power from 700 to 1000 W for 10 min; (2) maintenance of microwave power at 1000 W for 10 min and (3) cooling at zero microwave power for 15 min. Consequently, one digestion run lasted 35 min. Because of the limited supply of sample material for skin analyses, only 50 mg of pooled material was used. To check for possible contamination of reagents and vessels, a blank was run with each series of standard reference materials or samples.

After digestion, blank solutions and samples (reference materials and woodlice) were transferred to 50-mL glass vessels which were filled to the mark with ultrapure water (Millipore, Eschborn, Germany). The final solutions were then analyzed by ICP-OES for the elements under consideration.

Analyses by ICP-OES

Analyses were carried out by ICP-OES (Optima 3300 DV, Perkin Elmer, Norwalk, CT), equipped with a 40-MHz, free-running RF generator and an array detector allowing for the simultaneous determination of the elements using four different wavelengths. A two-point calibration at 0.25 and 2.50 mg/L for each element was carried out using a multielement standard solution (Spex Certiprep, Metuchen, NJ). The instrumental operating parameters and wavelengths used are summarized in Table I.

RESULTS AND DISCUSSION

This section is divided into two parts. The first part, devoted to quality control, details the selection and use of wavelengths for analysis by ICP-OES, the limits of detection

and quantification, and, finally, the results of the analyses of the reference materials. The second part details the application of the analytical procedure to woodlouse samples (total animal and skin of animals).

Quality Control

Selection and Use of Wavelengths

Wavelengths were selected according to the literature [7–9]. Although detection of emission in ICP-OES is element-specific, interference may occur if the optical system of the spectrometer does not fully separate two emission lines which are very close to each other [9]. In case the interferent reveals numerous emission lines and its concentration in the sample is much higher than that of the analyte, it may be difficult to find an appropriate wavelength for analysis. Fe, for example, with more than 400 ICP emission lines [10], may have a severe impact on the correct determination of Mn [11]. Although there are mathematical models like Multi-Spectral Fitting which help overcome spectral interferences by constructing the spectrum of an undisturbed analyte [12], switching to another wavelength, free of interference, is a better solution.

To determine which of the recommended wavelengths were best suited for the analyses of the woodlice and their skins, the four wavelengths for each element, listed in the lower part of Table I, were used in the analyses of reference materials.

Limit of Detection and Limit of Quantification

The limits of detection (LOD) and quantification (LOQ) are summarized in Table II. They were obtained by replicate analyses of 10 blanks, consisting of 3 mL of 65% suprapur HNO_3 made up to a volume of 50 mL by ultrapure water. LOQ values account for the final volume of the sample (weight 100 mg) after digestion and dilution. Similar LODs and, consequently, LOQs of $<1000\,\mu g/L$ for all wavelengths were obtained for Fe and Mn only. In the case of Zn, at least three wavelengths showed

TABLE II Limit of detection (LOD) and limit of quantification (LOQ[a]) for each element at different wavelengths

Element	*Wavelength* (nm)	*LOD* (μg/L)	*LOQ* (μg/L)
Al	237.313	8.0	4000
	308.215	15.7	7850
	309.271	37.4	18 700
	396.152	0.8	400
Fe	234.349	1.7	850
	238.204	0.8	400
	239.562	0.7	350
	259.940	0.3	150
Mn	257.610	0.3	150
	259.372	0.1	50
	260.568	0.4	200
	293.305	0.8	400
Zn	202.548	0.9	450
	206.200	0.9	450
	213.857	0.5	250
	334.501	18.0	9000

[a]LOQ accounts for digestion and dilution.

similar sensitivities, whereas the fourth was remarkably less sensitive (LOD 6–18 μg/L). For Al, only one wavelength with an LOD < 1 μg/L was found. The data in Table II indicate that certain wavelengths were inappropriate for the analysis of trace amounts due to insufficient sensitivity (Al 308.215, Al 309.271, Zn 334.501).

Analyses of Reference Materials

Results of the analyses of Al, Fe, Mn and Zn in Dorm-2, Dogfish muscle (*S. acanthias*), and of Fe, Mn and Zn in SRM 1577b Bovine Liver using four different wavelengths for each element are compiled on the right-hand sides of Table III (Dorm-2) and Table IV (Bovine Liver), respectively. The mean values of six subsequent analyses are indicated. The left-hand sides of the tables list the certified concentrations and the tolerance levels as indicated by the manufacturers of the reference materials.

The reference materials were selected to respond to the following requests:

- to match the animal matrix as closely as possible;
- to contain the elements under consideration in a concentration range which was both expected in the animals and also accessible to ICP-OES after digestion and dilution;
- to contain certain elements in both standards for double control (Fe, Mn, Zn).

The reference material should allow the whole analytical procedure to be assessed. Moreover, matrix reference materials which usually contain the analytes of interest in their natural form and in their natural environment should be chosen to yield a matrix that closely resembles the matrix of the samples to be analyzed. In addition, they should ideally contain analytes with well-characterized reference values which are similar to the samples to be tested [13]. Matrix reference materials can be introduced at the beginning of the analytical process to assess the quality of the whole process, including sample preparation (i.e. sample digestion), as well as the final analysis

TABLE III Dorm-2, Dogfish muscle (*Squalus acanthias*; National Research Council, Canada) certified concentrations and means of analyses ($N = 6$) for different wavelengths, and tolerance based on the 95% confidence interval for the mean

Element	*Certified concentration* (mg/kg)	*Tolerance* (mg/kg)	*Element*	*Wavelength* (nm)	*Analysis* (mg/kg)	*S.D.* (mg/kg)
Al	10.9	1.7	Al	237.313	11.6	1.2
				308.215	14.5	2.1
				309.271	n.a.	
				396.153	12.1	0.9
Fe	142	10	Fe	234.349	132.7	5
				238.204	136.0	6
				239.562	136.8	7
				259.940	135.9	5
Mn	3.66	0.34	Mn	257.610	3.73	0.13
				259.372	4.01	0.18
				260.568	3.72	0.12
				293.305	3.70	0.12
Zn	25.6	2.3	Zn	202.548	27.4	0.3
				206.200	27.1	0.4
				213.857	26.6	0.4
				334.501	26.9	0.7

S.D.: Standard deviation; n.a.: no results available (for reasons, see text).

TABLE IV SRM 1577b, Bovine Liver (National Institute of Technology, USA) certified concentrations and means of analyses ($N = 6$) for different wavelengths, and tolerance based on the 95% confidence interval for the mean

Element	*Certified concentration* (mg/kg)	*Tolerance* (mg/kg)	*Element*	*Wavelength* (nm)	*Analysis* (mg/kg)	*S.D.* (mg/kg)
Fe	184	15	Fe	234.349	181	1.7
				238.204	187	2.5
				239.562	186	3.3
				259.940	187	3.0
Mn	10.5	1.7	Mn	257.610	10.9	0.05
				259.372	10.7	0.15
				260.568	10.8	0.06
				293.305	10.7	0.10
Zn	127	16	Zn	202.548	135	1.9
				206.200	133	1.7
				213.857	127	1.9
				334.501	136	2.0

S.D.: Standard deviation; n.a.: no results available (for reasons, see text).

(e.g. sample transport, nebulization, atomization). By comparing the result obtained using the analytical method with the value of the reference material, any bias in the result can be detected, and if necessary, appropriate corrective action can be taken. If the matrix of the reference material resembles the matrix of the routine samples as closely as possible, all problems that might appear during sample preparation (e.g. completeness of matrix dissolution) and analysis (e.g. spectral interferences) are well replicated by the reference material [14]. If, under these circumstances, a result for the reference material is obtained that agrees with the certified concentration, this provides good evidence that the results on the routine samples are appropriate.

Animal matrix often contains considerable amounts of organic matter (e.g. fat, proteins). According to the manufacturer, the fat content in Dorm-2 is about 5% (in the case of Bovine Liver, the fat content is not given). Organic matter, if not completely destroyed, may have a severe impact on the recovery of trace metals [15,16].

Unfortunately, many data referring to metal concentrations in woodlice, e.g. [4,5], are based on reference materials like SRM 1573a Tomato Leaves, or SRM 2710 Montana Soil, where there is little or no similarity between the matrix of the reference material and the samples. The use of SRM 1577b Bovine Liver is even more advisable, as some elements are reported to be stored in the digestive organs of the woodlice [3].

The results from Table III show that not all of the wavelengths used were suitable for the analysis of Dorm-2. As already expected from the determination of the LODs, Al 309.271 was not sufficiently sensitive to determine the element in the sample. In the case of Al, only two wavelengths, Al 237.313 and 396.153, provided results that agreed with the certified value. The concentration measured for Al 308.215 is considerably above the tolerance level of the SRM, which is mainly due to the insufficient sensitivity of this wavelength for the reliable determination of traces of Al in the digestion solution. The concentrations for Fe, Mn and Zn were within the tolerance levels of the certified values, although in the case of Mn, slight differences could be stated for some wavelengths: Mn 259.372 showed a concentration at the very top of the tolerance level, whereas the other concentrations were very close to the certified value. There was, however, no indication of severe spectral interferences for any of the wavelengths used.

The data in Table IV (Bovine Liver) show that for Fe, Mn and Zn, all wavelengths used yielded results that were in good agreement with the certified values.

Summing up the results presented above, a reliable analysis of Al, Fe, Mn and Zn in Dorm-2, Dogfish muscle, and of Fe, Mn and Zn in SRM 1577b Bovine Liver, was possible on at least two (in most cases, three) wavelengths for each element. Analyses at these wavelengths were free from spectral interferences and yielded results which were in good agreement with the certified concentrations. From this and the fact that the two reference materials used for method development matched the matrix of the woodlice samples very closely, we concluded that for the routine analysis of the woodlice samples, the use of only the most sensitive of the appropriate wavelengths would lead to reliable analytical results in these samples as well.

Analyses of Woodlice Samples (Total Animals and Skins)

Tables V and VI list the results of the analyses of the total animals and the skins of *P. scaber* (Table V) and *P. dilatatus* (Table VI) using the most sensitive of the appropriate wavelengths. These are indicated in the second column of each table. For each element, the mean of three analyses, including the standard deviation, is indicated. As can be seen from the data, the concentrations of the same element vary considerably between animal species, except for the concentration of Mn, which is very similar for both species. This variation is surprising since both species had grown up under standardized and almost identical conditions. In particular, *P. dilatatus* seemed to accumulate Al and Fe much more efficiently than *P. scaber*, since the concentrations of these elements in *P. dilatatus* were almost three times higher than in *P. scaber*. Both elements are ubiquitous. The concentration of Fe in vegetables is relatively high, with an average value of 15 mg/kg (fresh mass) [17,18]. Maximum concentrations, however, can be much higher, especially if values refer to dry mass. In dried SRM 1573a, Tomato Leaves, the certified Fe concentration is 368 ± 7 mg/kg.

TABLE V Elemental concentrations in *Porcellio scaber* (total animal and skins)

Element	*Wavelength* (nm)	*Total animal* (mg/kg)	*S.D.* (mg/kg)	*Skins* (mg/kg)	*S.D.* (mg/kg)
Al	396.152	229	0.5	631	6.4
Fe	259.940	249	1.4	502	2.1
Mn	259.372	77.6	0.4	142	0.8
Zn	213.857	170	0.8	28.2	1.1

$N = 3$; S.D.: standard deviation.

TABLE VI Elemental concentrations (mg/kg) in *Porcellio dilatatus* (total animal and skins)

Element	*Wavelength* (nm)	*Total animal* (mg/kg)	*S.D.* (mg/kg)	*Skins* (mg/kg)	*S.D.* (mg/kg)
Al	396.152	621	2.1	922	6.3
Fe	259.940	642	7.1	969	7.0
Mn	259.372	77	1.4	125	1.3
Zn	213.857	97.6	0.5	32.3	0.5

$N = 3$; S.D.: standard deviation.

The concentration of Zn in *P. scaber* was found to be in good agreement with that published by Hopkin [3], who indicated a value of 186 ± 21 mg/kg. However, he found an Fe concentration in these animals that was considerably lower (110 ± 12 mg/kg) than the value we found. The better precision of our results may be because the woodlice we analyzed had grown up under standardized conditions resulting in a more homogenous distribution of elements. Unfortunately, data on metal concentration in uncontaminated woodlice are lacking. As mentioned earlier, the animals are frequently used to monitor heavy-metal pollution [1,2]. For a reliable risk assessment based upon bioindication by woodlice, more data on metal concentrations in uncontaminated animals are needed. So far, many studies are based on the determination of a so-called 'lowest-observed-effect concentration' following exposure to artificially contaminated food, which is also used to examine the possible loss of heavy metals by woodlice [5].

We examined another possible way of decontamination of isopods from heavy metals: moulting. Column 5 in Tables V (*P. scaber*) and VI (*P. dilatatus*) summarizes the elemental concentrations we obtained when analyzing the skins of the animals. As can be seen, Al, Fe and Mn were considerably enriched in the skins compared with their concentration in the total animal. The enrichment factors for the elements were: Al (2.8/1.5), Fe (2.0/1.5) and Mn (1.8/1.6), the first number referring to *P. scaber* and the second to *P. dilatatus*. While the enrichment of Fe and Mn was almost the same in both animal species, skin enrichment of Al in *P. scaber* seemed to be more efficient than in *P. dilatatus*. In contrast, the concentration of Zn in the skins diminished remarkably compared with that for the whole animal. It decreased to about 17 and 33% of its original concentration in *P. scaber* and *P. dilatatus*, respectively. This appeared reasonable, as some heavy metals have been found to be stored in lysosomal vesicles of the hepatopancreas of the animals [3].

Although the present data are far from sufficient to make a final statement, mainly due to the limited number of samples and analyses, they at least provide a substantial hint that moulting in woodlice could be a possible method of Al, Fe and Mn decontamination, although this mechanism is obviously not appropriate for Zn. Further studies are needed to determine whether moulting is really a substantial means of trace-element decontamination in woodlice.

CONCLUSION

The reliability of Al, Fe, Mn and Zn determination in *P. scaber* and *P. dilatatus* and their skins has been shown. The completeness of the sample digestion and the quantitative recovery of the elements under consideration were checked using two reference materials of animal origin to match the matrix of samples and reference materials as closely as possible. Moreover, standard reference materials were analyzed using different wavelengths for each element to check for possible spectral interferences. Concentrations of the elements were in agreement with the certified values for most of the wavelengths used. Despite the complex matrix of the animal reference materials, no spectral interferences were observed. The method was applied to uncontaminated woodlice samples and results compared with data from the literature. In an additional step, skins of the animals were analyzed and results compared with the original concentration in the animals. While the Zn concentration in the skins had considerably

decreased, Al, Fe and Mn enrichment was observed. Moulting was assumed to be a possible method of decontamination for these elements.

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