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Micro-heterogeneity of trace elements in reference materials - determination and statistical evaluation

U. Kurfürst[1], J. Pauwels[2], K.-H. Grobecker[2], M. Stoeppler[3], and H. Muntau[4]

[1] University of Fulda (Fachhochschule), Department of Nutrition, P.O. Box 1269, W-6400 Fulda, Federal Republic of Germany
[2] Commission of the European Communities, Central Bureau of Nuclear Measurements (CBNM), B-2440 Geel, Belgium
[3] Forschungszentrum Jülich (KFA), Institute for Applied Physical Chemistry, W-5170 Jülich, Federal Republic of Germany
[4] Commission of the European Communities, Joint Research Centre, Environmental Institute (EI), I-21020 Ispra (VA), Italy

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Summary. Solid sampling graphite furnace ZAAS is very efficient for homogeneity testing because of the low sample intake with this method. A number of biological reference materials were investigated for heterogeneity with respect to the laboratory sampling error. It was observed that in some cases the investigated material appears to contain a distinct particle fraction of much higher trace element content ("nuggets") than in the average particles. It could be shown theoretically and experimentally that in the case of nuggets in a material, statistical evaluation using a Poisson probability function was necessary to obtain realistic values for heterogeneity. The measured homogeneity constants for certain elements in each material give an excellent way of calculating the minimum sample mass necessary for reliable element determination. It is proposed to include this testing method in the certification campaign of new CRMs.

1 Introduction

Homogeneity is considered to be the most vital pre-requisite for a candidate reference material, and therefore related testing methods deserve great attention. Environmental reference materials, such as soils, sediments, waste materials and even plant and tissue materials typically consist of many different solid phases with characteristic physical properties such as size, density and geometrical form and widely varying contents of trace elements, which in some cases may cover four orders of magnitude [1].

Homogenisation of such complex mixtures to such a degree, that replicate subsamples of the size requested by modern trace analytical techniques, i.e. approximately 100 mg, does not show significant differences within the limits of the precision of the testing method, might be considered an art rather than a science.

It had been observed that conventional homogeneity testing, as introduced into candidate reference material production practices [1, 2] can overlook micro-heterogeneities in otherwise perfectly homogenised materials and cause serious problems. With luck these heterogeneities may be detected during certification analysis as encountered during the certification of a series of meat materials for metal contents [3]. Pig kidney, bovine liver and bovine muscle were tested and occasionally high lead contents, well above the average level, were found at low subsample sizes. Detailed investigation showed that few, but rather large cleavage fragments of calcium oxalate, just below the 125 μm particle size, occurred in the sample and that these crystals contained approximately 1% of lead. Such micro-heterogeneities would escape detection by classical homogeneity testing methods due to the relatively high sample intake requirements. Solid Sample Zeeman Atomic Absorption Spectrometry (SS-ZAAS) with a graphite furnace operating at a submilligram sample intake level, shows correspondingly enhanced detection power for the identification of particles carrying extremely high analyte contents [4].

2 Experimental

The analyses have been carried out with the Zeeman Atomic Absorption Spectrometer SM 20 (GRÜN-Analysengeräte Wetzlar, Germany). The instrument is specially designed for the direct introduction of solid samples [5]. A large number of replicates were carried out mainly by the use of an automated sampler for powdered samples which was described recently [6].

The methodology of solid sampling with GF-AAS has been described in detail [7]. The calibrations were performed with different CRMs. Because only the precision (scatter) of the results plays a role in this investigation, no special attention was paid to overcome slight systematic errors caused by different calibrant and sample materials. The results are normalised so that the mean value is unity in all cases.

The Reference Materials which are used for this homogeneity study were certified by the European Community Bureau of Reference (BCR) [3, 8, 9] or by the National Institute for Environmental Studies (NIES) (Japan) [10]. Two Reference Materials from the German Speciman Bank (GSB) are also used [11].

The SS-ZAAS data were checked for long term drift and if necessary corrected so that the regression of the content values on subsample terms remains constant. For all statisti-

Correspondence to: U. Kurfürst

cal calculations and graphics the program "STATGRAPHICS" (V 2.6) of the STSC Inc. was used.

3 Statistical considerations

The concept of the *sampling error* s_E of an analyte element E in a powdered sample (i.e. the deviation of the distribution of an analyte in samples drawn from a bulk material), was discussed by Wilson in 1964 [12], and can be represented in a simplified form (only two species and uniform sized particles) by:

$$s_E = (c_1 - c_2) \cdot \sqrt{\frac{\varrho_1 \cdot \varrho_2}{\varrho^2}} \cdot \sqrt{\frac{p_1 \cdot p_2}{N}} \cdot \frac{1}{\sqrt{m}} \quad (1)$$

In this equation:

p_1 and p_2 = *portions (mass fraction) of species 1 and 2 in the sample*

c_1 and c_2 = *content (mass fraction) of the analyte in these species*

ϱ_1 and ϱ_2 = *density of the materials of species 1 and 2*

ϱ = *sample density*

N = *number of particles per unit mass of sample*

m = *mass of subsamples*

The probability of obtaining these species in subsamples follows a binominal distribution. For a large number of particles, this distribution closely approximates a normal distribution.

In 1966 Gy [13] published a more practical concept, where the sampling error was described by different factors (particle shape, particle size, degree of homogeneity and the chemical composition), which can be useful in controlling the reduction of mineralogical field samples to laboratory size. The application of these equations is, however, difficult as the internal physical sample properties for pulverised biological samples are not easy to assess quantitatively.

In 1973 Ingamells and Switzer [14] introduced the *sampling constant* K_s, which includes all internal sample properties determining the heterogeneity:

$$RSD = \sqrt{K_s/m} \quad (2)$$

In this equation the relative standard deviation (RSD) of analytical results is used to calculate the sampling constant K_s. It represents the subsample mass necessary to ensure a relative subsampling error of 1% (65% confidence level in case of a normal distribution), on the condition that the analytical procedure is free of analytical error.

However in general, random errors due to the analytical procedure dominate the overall standard deviation, or are not accurately known, so that a variance analysis cannot be performed to isolate the *relative sampling error (RSE)* using:

$$RSE^2 = RSD^2 - \Sigma\ (rel.anal.std.err.)^2 \quad (3)$$

With the advent of SS-ZAAS, the determination of sampling constants of trace elements, however, became easier; for sub-milligram samples it is generally true that the spread in the results is mostly dominated by the heterogeneous distribution of the analyte in the material. Only the within-run variation in the instrumental response, which is easy to determine, can give an additional contribution to the overall random error.

In this case the sampling error can be expressed as the *homogeneity constant* h_E as defined by Kurfürst et al. [15]:

$$s_E = h_E / \sqrt{m} \quad (4)$$

At a certain level of subsample mass, the distribution of analytical results may deviate significantly from normal [4, 14, 16]. This is especially the case when a material contains a very small number of grains with extremely high analyte content ("nuggets") compared to the main material. In this case, results must be statistically treated using the Poisson probability function, where the *probability P* of obtaining a number of nuggets *(x)* in a subsample can be calculated by:

$$P(x) = \frac{z^x \cdot e^{-z}}{x!} \quad (5)$$

where:

x = *number of nuggest in a subsample of mass m;*

z = *average number of nuggets in subsamples of mass m.*

With this type of distribution, $\sqrt{z}$ represents the standard deviation of the number of nuggets in a large set of subsamples.

The total content c and the sampling error s_E corresponds to (s. Fig. 1a-1c):

$$c = z \cdot c_n + c_B \quad (6)$$

$$s_E = c_n \sqrt{z} \quad (7)$$

where:

c_n = *contribution of one nugget to the content with subsamples of mass m;*

c_B = *basic content of the sample (without nuggets).*

If z is smaller than 1, the distribution is only right-sided because samples with no nugget (x = 0) are most probable. If z is larger, then the probability function is two-sided, but skewed, with a tail to larger x-values. With increasing z the probability function becomes more and more symmetric. From z = 9 the Poisson distribution reaches the shape of a normal distribution. Figure 1 gives some examples of the Poisson probability mass function.

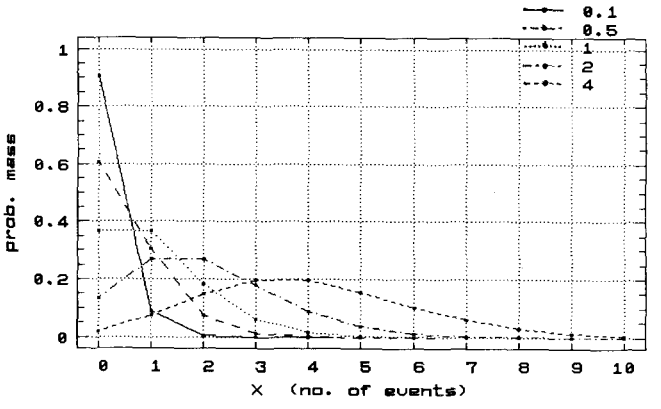


Fig. 1. Poisson probability mass functions with different mean values (z = 0.1, 0.5, 1.0, 2.0, 4.0)

An illustration of the nugget effect is given in Fig. 2 by simulated analytical results (2a), the statistical evaluation following the Poisson distribution for the nuggets (2b) and the influence of a larger sample mass on the nugget distribution and the spread of results (2c).

For nugget containing samples the homogeneity constant obeys [Eqs. (4) and (7)]:

$$h_E = c_n \sqrt{z \cdot m} \tag{8}$$

Both, c_n and z, depend on the subsample mass m. However this equation can be transformed into terms which are independent of m by writing Eq. (8) in the form:

$$h_E = c_n \cdot m \sqrt{z/m} \tag{9}$$

where the *number of nuggets per unit mass z/m* and the *contribution of one nugget for samples of unit mass* $c_n \cdot m$ are constant for a given nugget-containing sample.

Considering Eq. (6) and the fact that z can be expressed by the *portion of the nugget material fraction* p_N by $N \cdot p_N \cdot m$, one can express the homogeneity constant as:

$$h_E = (c - c_B) \cdot \frac{1}{\sqrt{N \cdot p_N}} \tag{10}$$

And considering that:

$$c - c_B = a \cdot p_N \cdot c_N \tag{11}$$

where:

c_N = *content of the nugget material*

a = *ratio of the densities of the nugget and the basic material*

Eq. (4) becomes:

$$s_E = c_N \cdot \sqrt{\frac{a \cdot p_N}{N}} \cdot \frac{1}{\sqrt{m}} \tag{12}$$

The sampling error calculated from the nugget model [Eq. (12)] corresponds exactly to Eq. (1), if one considers the "nugget conditions" ($\text{species}_1 = \text{Nuggets}_N$, $\text{species}_2 = \text{basic material}_B$, $\varrho_N = a\varrho_B$):

$$c_N >> c_B \quad p_N << 1 \quad \varrho \cong \varrho_B \tag{13}$$

For practical use and to compare different materials, the *relative homogeneity constant* H_E is more appropriate:

$$H_E = \frac{h_E}{c} \cdot (100\%) \tag{14}$$

so that Eq. (4) becomes:

$$RSE = H_E \cdot \frac{1}{\sqrt{m}} \tag{15}$$

The relative homogeneity constant H_E represents the relative sampling error if a subsample of unit mass is used. It corresponds to the square root of the sampling constant K_s. The use of H_E is more convenient for analytical purposes, when the sampling error is significantly different from 1%.

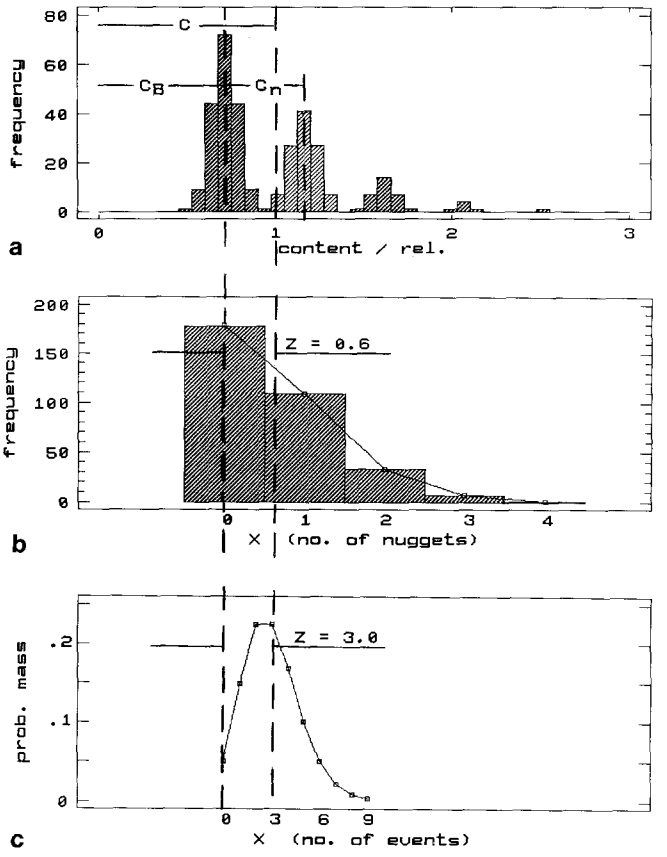


Fig. 2. Simulated analytical data and evaluation of an "ideal" nugget effect. **a** Frequency histogram with a basic fraction of the content c_B and fractions with different numbers of nuggets. The contribution of one nugget to the content c_n is chosen so large, that the fractions are totally separated, despite of a slight broadening effect by random analytical errors. **b** The distribution of the number of nuggets x in all subsamples of Fig. 2a and the Poisson fit which gives an average number of nuggets in all subsamples of $z = 0.6$. The scale of the figures is chosen so that the nugget fractions and the means (c and z) of both types of distribution are adjusted on the same vertical line, so Eq. (6) can be read directly from these figures. **c** Poisson probability mass function with an average of $z = 3$. This corresponds to a five times higher sample mass. Although higher numbers of nuggets are probable, the decreased contribution of one nugget has a stronger influence [s. Eq. (7)], so that the scatter of results is smaller.

There are three possibilities of determining the degree of heterogeneity as a property of a given sample:

1. If all internal properties of the sample are known or can be determined, the Wilson equation (1) or the concept of Gy can be used [for nugget-containing samples Eq. (12)]. An example is given in [15] for a pulverised total wheat sample.

2. If an analytical method is available where all random errors can be determined, the sampling error s_E can be calculated by an analysis of variance from a sufficiently large set of analytical results. Then H_E (K_s) can be calculated using Eq. (15) [17, 18]. An example is given in this paper (Zn in Cod Muscle).

3. If the sample mass can be reduced to such an extent that the nugget effect appears (i.e. in practice $z < 4$), then c_n and z can be determined. So the homogeneity constant can be calculated using Eq. (8). The advantage of this method is that no "broadening effect" due to random analytical or instrumental errors affect these values and no information about the internal parameters is needed. Like [4], this paper represents mainly a "worse case study", which focuses on an investigation of the rare samples which are found showing this kind of micro heterogeneity effects.

4 Results

a) Normally distributed results

As a test material for reproducibility and contamination-free operation of the sampling procedure BCR CRM-150 (Milk Powder) was used. Figures 3a and 4a show histograms of the SS-ZAAS data. The results are normally distributed as clearly shown by the normal probability plots in Figs. 3b and 4b.

In this case the analytical error is mainly caused by baseline noise, because the determinations were carried out near the limit of detection with subsample masses between 0.2–0.4 mg. Blind measurements (no sample loading) gave a standard deviation of the same order. For this sample the relative homogeneity constants H_{Cd} and H_{Pb} are $< 1\ mg^{1/2}$.

Figures 5a, b, and c show three series of zinc determinations in BCR CRM-422 (Cod Muscle) with different subsample masses. With increasing sample mass the standard deviation decreases. If the RSD is reduced to the RSE [Eq. (3)] by considering the contribution of the noise scatter, a fit of RSE over the subsample mass with the regression function of Eq. (15) gives a relative homogeneity constant of $H_{Zn} = 3.8\ mg^{1/2}$ (Fig. 6).

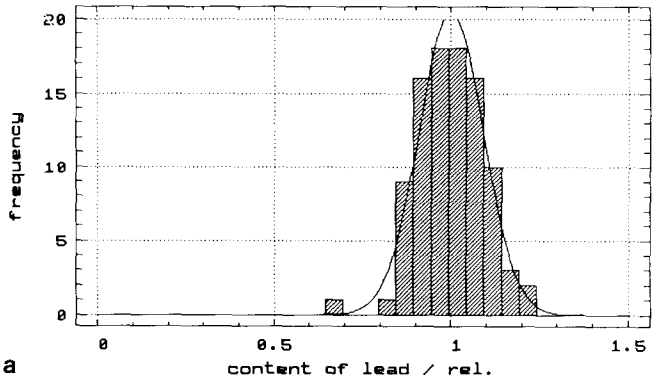
This is the normal case in biological materials, where no material fraction with an extremely high analyte content exists and/or the different mass fractions are so large that there are many particles ($z > 9$) of every material fraction in each subsample.

b) Skew distributions

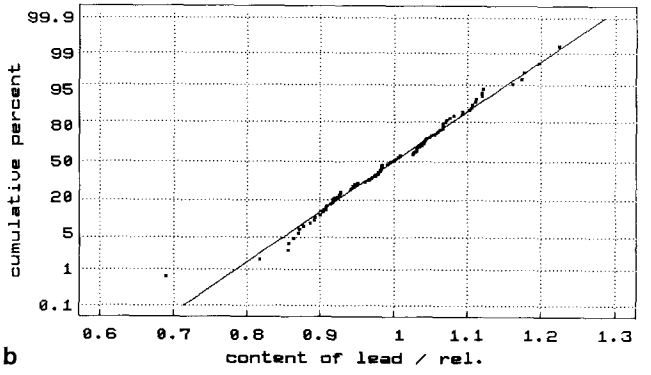
If a small particle fraction with high analyte content exists in the sample, i.e. any subsample contains only a few of these nuggets ($z < 9$), an asymmetrical distribution of results appears.

Figure 7a shows a histogram of a lead determination in NIES No. 6 (Mussel) with a subsample mass of $m = 0.25$ mg. The distribution is skewed towards the larger contents. The class width was chosen in such a way that the shape of a Poisson distribution can be recognised. The columns can be interpreted as subsamples with x particles of high analyte content (nuggets), where fractions with $x = 0$ to $x = 3$ are very probable and fractions with $x = 4$ to $x = 6$ appear only rarely. In Fig. 7b the Poisson probability mass function for $z = 1.2$ is plotted, which has a similar shape.

Figure 8a shows a similar distribution of a lead determination in GSB RM 1 (Spruce Shoot). The broad almost rectangular shaped mode on the left side indicates a Poisson distribution with an average of approx. 1, in which the probability of $x = 0$ and $x = 1$ is about equal. Also the steps in the normal probability plot (Fig. 8c) show that two fractions are superimposed. In Fig. 8a the designated fraction with one

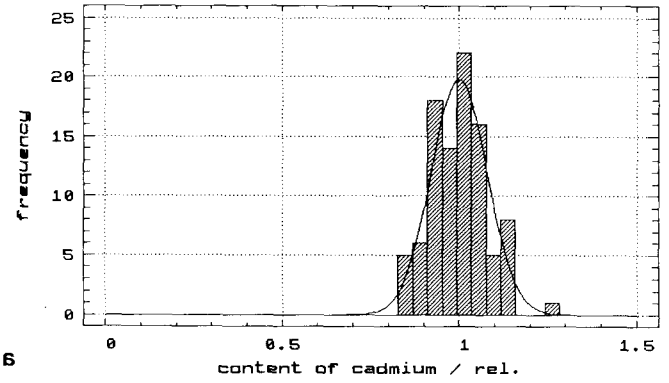


a



b

Fig. 3. Normalised SS-ZAAS data from a lead determination in BCR CRM-150 (Spiked Skim Milk Powder) **a** Histogram and normal distribution fit; **b** Normal probability plot



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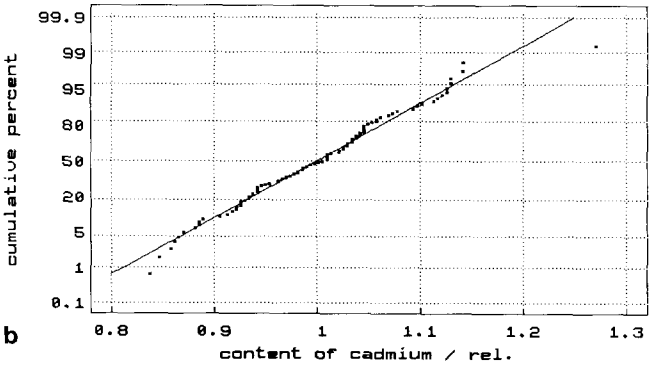

b

Fig. 4. Normalised SS-ZAAS data from a cadmium determination in BCR CRM-150 (Spiked Skim Milk Powder) **a** Histogram and normal distribution fit; **b** Normal probability plot

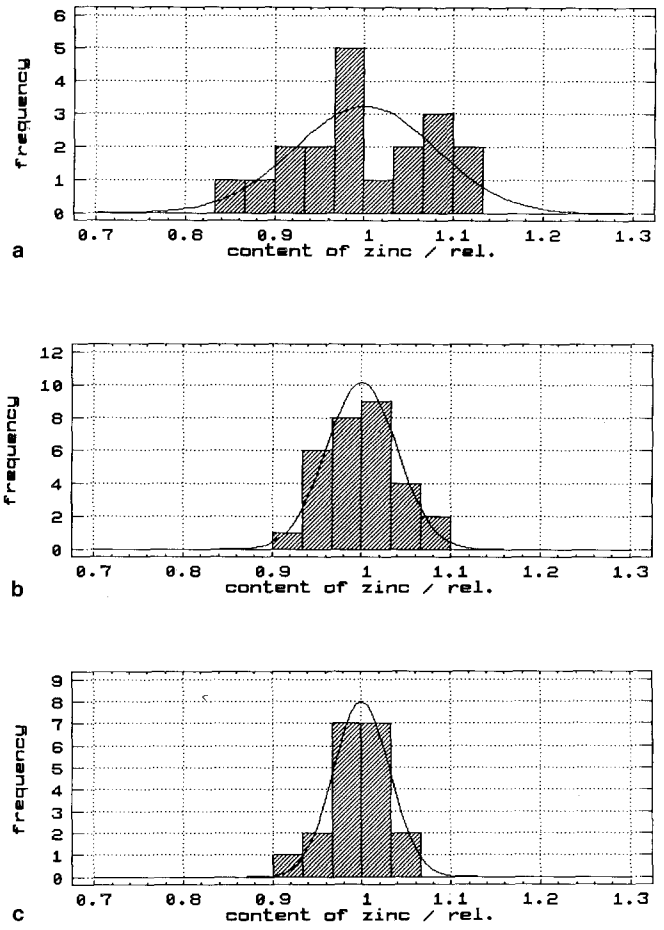

Fig. 5. Frequency histograms and normal distribution fits of normalised SS-ZAAS data from a zinc determination in BCR CRM-422 (Cod Muscle) for a subsample mass of **a** m = 0.35 mg; **b** m = 1.0 mg; **c** m = 1.9 mg

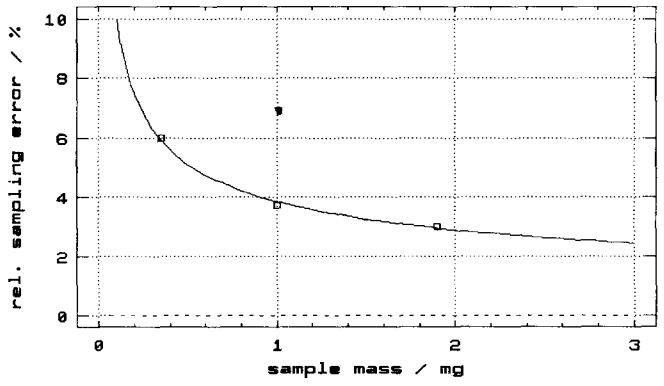

Fig. 6. Regression of the relative sampling error on sample mass of the SS-ZAAS data from Fig. 5 with the function RSE = $3.8/m^{1/2}$

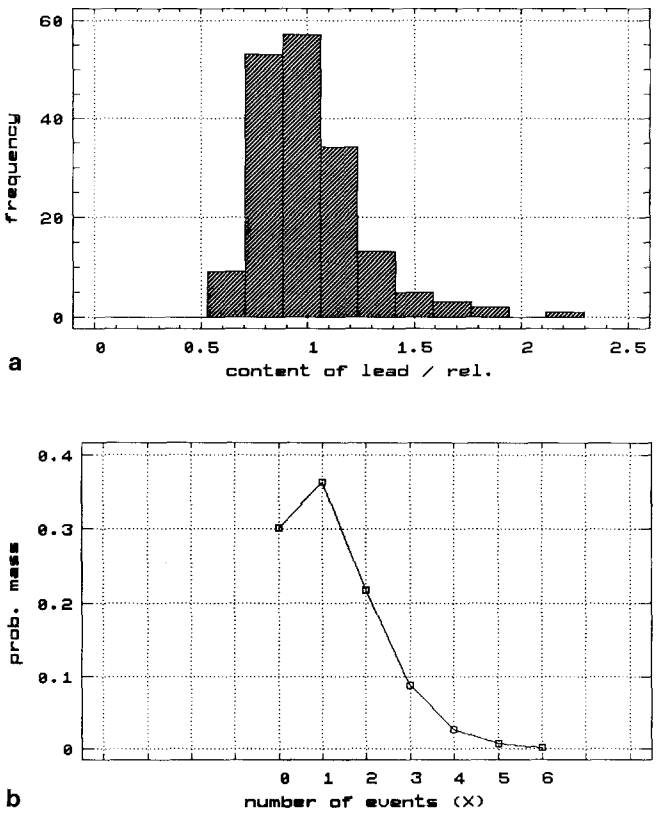

Fig. 7. Normalised SS-ZAAS data from a lead determination in NIES No. 6 (Mussel Tissue) **a** Frequency histogram; **b** Poisson probability mass function with a mean of z = 1.2

nugget is hatched with a different pattern. The distribution of the fraction with no nugget is always supposed to be normally distributed (Figs. 8a, 9a, 10a, 11a, b).

This partition considers a broadening effect. The tail towards higher contents indicates that the nugget size distribution is asymmetrical. Thus the shape of the fraction with one nugget must be superimposed on the nugget size distribution. Joining the values in columns with equal numbers of nuggets a Poisson probability fit of z = 0.82 can be achieved (Fig. 8b).

c) multi modal distributions

Figure 9a shows the histogram of results of a cadmium determination in BCR CRM-184 (Bovine Muscle). It is highly skewed, but moreover a distinct second maximum (mode) appears. In this case the contribution of one nugget to the total content c_n is so large that with the used subsample mass (m = 0.65 mg) the different nugget fractions are separated.

A Poisson distribution fit for the nuggets in the subsamples of z = 0.46 is possible, when a broadening effect by the nugget size distribution is taken into account. The two fractions of zero and one nugget can be identified with the normal probability plot (Fig. 9b) as two segments with different slopes.

Another example of this effect is shown in Fig. 10a for the determination of copper in a GSB RM (Herring-Gull Egg). The nugget fraction is very small, so the average number of nuggets in subsamples of mass m = 1.3 mg is only z = 0.12. The separation of the fraction with one nugget is facilitated by using the normal probability plot (Fig. 10b), which shows two slopes and small steps in the overlapping region.

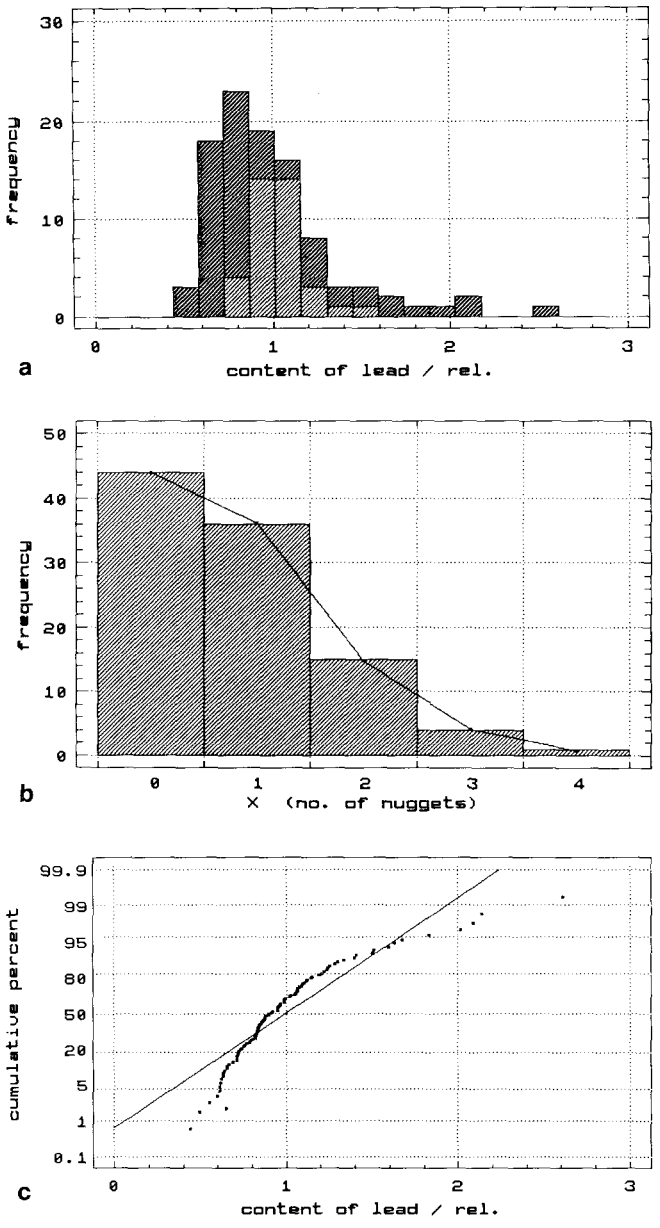

Fig. 8. Normalised SS-ZAAS data from a lead determination in GSB-RM 1 (Spruce Shoot) **a** Frequency histogram; **b** Distribution of the nuggets and Poisson fit (z = 0.82); **c** Normal probability plot

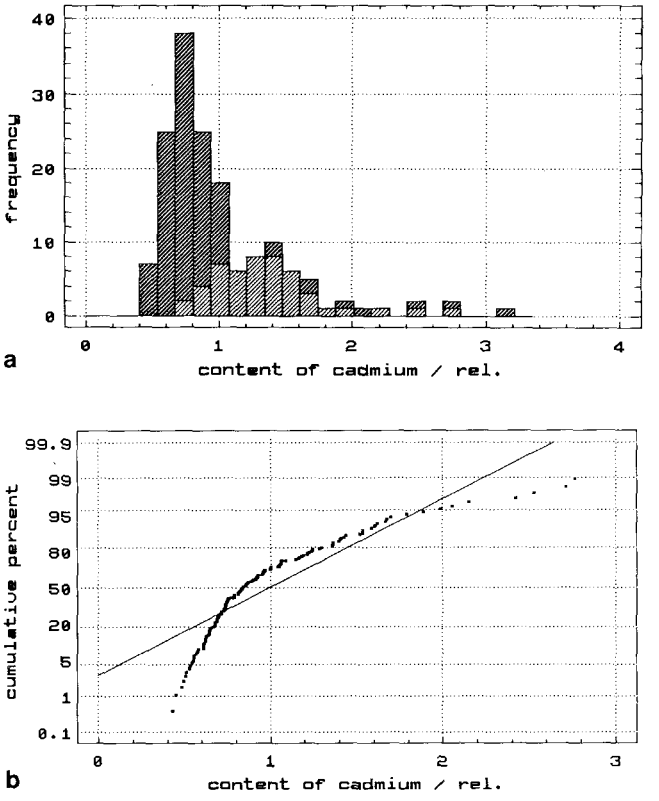

Fig. 9. Normalised SS-ZAAS data from a cadmium determination in BCR CRM-184 (Bovine Muscle). **a** Frequency histogram; **b** Normal probability plot

An almost complete separation of the nugget fractions is seen in Fig. 11 a for the determination of cadmium in BCR CRM-422 (Cod Muscle) for a subsample mass m = 0.6 mg. An average number of nuggets z = 0.39 was determined (Fig. 11 c).

If the subsample mass is increased, the contribution of one nugget to the total content becomes smaller and the average number of nuggets increases corresponding to the relation of the masses. Figure 11 b shows the histogram of an analysis of the Cod Muscle material with a subsample mass of m = 1.1 mg. The second maximum is shifted closer to the first one and the number of results with one nugget is larger, so that an average number of nuggets of z = 0.65 (Fig. 11 d) was found.

5 Discussion

The validity of the nugget model theory can be shown using the evaluated examples. Table 1 lists the experimentally determined and calculated values of the materials examined, including examples from previous papers.

The excellent agreement of the average values calculated from all (n) analytical results with the contents calculated by the nugget model [Eq. (6)] shows that reliable designation of the fractions of nugget numbers (x) is possible.

All examples show an overall RSD which is significantly larger than the RSE, calculated from the nugget model [Eq. (7)]. This reflects the fact that the RSD is also influenced by the instrumental response scatter (baseline noise), while, with the determination of the sampling error via the nugget model, none of the analytical and instrumental random errors have any influence!

Most important for the use of certified reference materials is that, even in the case of a large nugget effect, the RSE is small enough at subsample sizes of 200 mg; this mass is generally recommended as the minimum sample mass by CRM producers. The RSE thus gives only a small contribution to the analytical RSD; this is normally in the range of 5-10% [3].

With the demand for normally distributed results discussed by Pauwels et al. [19, 20], the portion of nugget material, p_N, is the decisive factor. If this portion is very small, a skewed distribution remains possible with larger subsample masses. Although Herring-Gull Egg (Cu) and Spruce Shoot

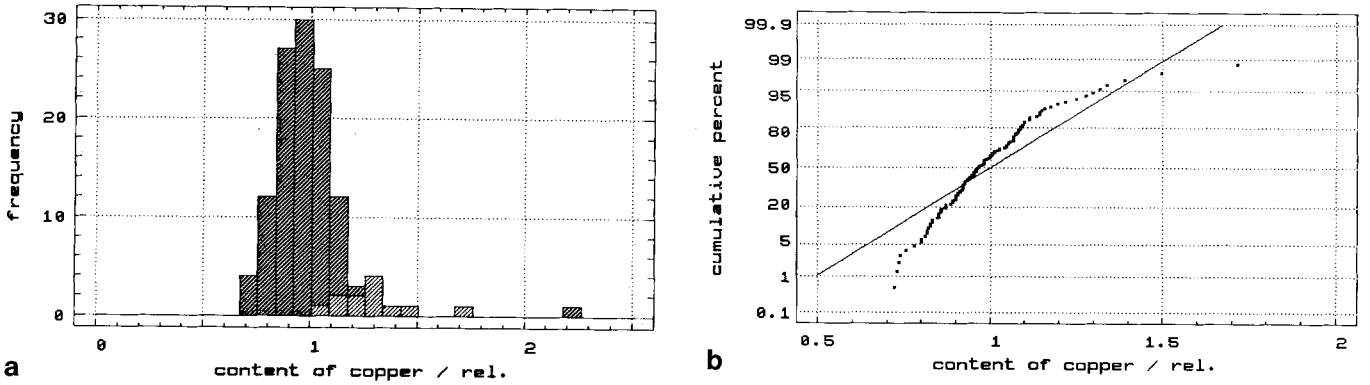

Fig. 10. Normalised SS-ZAAS data from a copper determination in a GSB-RM (Herring-Gull Egg). **a** Frequency histogram; **b** Normal probability plot

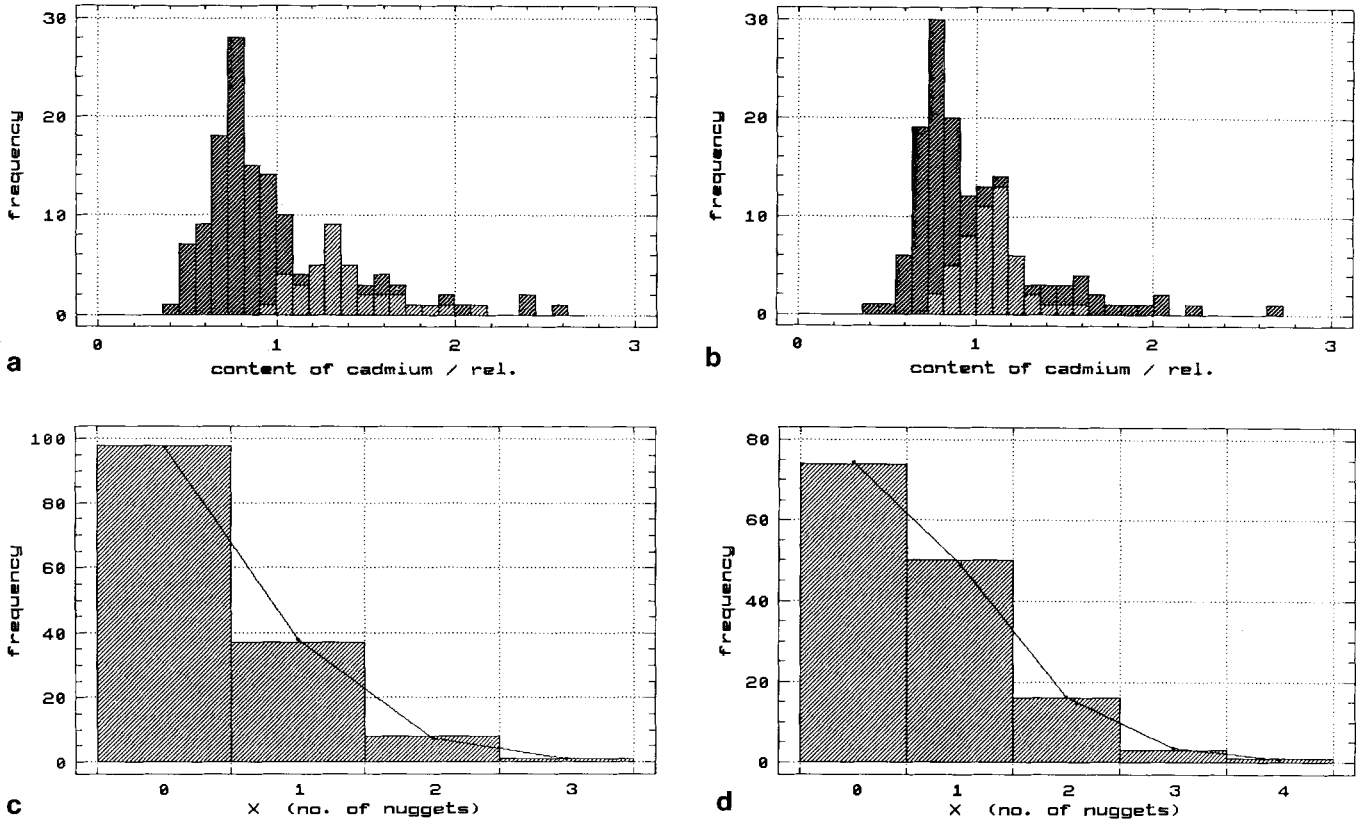

Fig. 11. Normalised SS-ZAAS data from cadmium determinations in BCR CRM-422 (Cod Muscle) **a** for a subsample mass of 0.6 mg and **b** for a subsample mass of 1.1 mg. **c** Distribution of the nuggets and Poisson fit ($z = 0.39$) of the determination in a. **d** Distribution of the nuggets and Poisson fit ($z = 0.65$) of the determination in b.

(Pb) have an identical homogeneity constant of 14 $mg^{1/2}$, the herring-gull egg material requires subsamples of approx. 100 mg to obtain normally distributed results, while for the spruce shoot material 3 mg is sufficient. This difference is caused by the difference in the z/m-value (0.09 and 3.2 mg^{-1}, (Table 1).

This effect can also be recognised with Cd in BCR CRM-422 and Pb in BCR CRM-184; in the latter the H-value is larger, but the subsample mass for normality is smaller. Both samples have approximately the same $c_n \cdot m$-value (0.36 and 0.34 mg) but a significant difference in the z/m-value (0.65 and 0.93 mg^{-1}).

Obviously the model of equal sized nuggets oversimplifies the constitution of real samples of biological origin. The particle sizes of ground and sieved powders cover a wide range, often several orders of magnitude for the diameter. It must be assumed, that the nugget particles also normally show such a size distribution.

Typical particle size distributions of ground materials are right sided (tail to large particles, often describable by a log normal distribution). Because the volume and the mass of the particles both increase with the third power of the diameter, the rare large particles contain the main portion of the analyte. These particles create the nugget effect, while the

Table 1. SS-ZAAS results and evaluation of nugget-containing reference materials

Reference material			Results and evaluation											
Identification	Element	Content[a] (mg/kg)	n	m (mg)	c_B (rel.)	c_n (rel.)	z	H_E[b] ($\sqrt{mg}$)	Subsample mass for Z=9 (mg)	5%RSE (mg)	1%RSE[c] (g)	RSE for m=200 mg (%)	Content[d] Content[e] (rel.)	Std. dev.[d] Samp. err.[e] (rel.)
Mussel (NIES No.6)	Pb	0.91	198	0.25	0.78	0.16	1.21	8.8	1.9	3.1	0.077	0.62	1.00 0.96	0.26 0.18
Spruce Shoot (GSB-RM 1)	Pb	3.1	100	0.26	0.73	0.28	0.82	13	2.9	6.8	0.17	0.92	1.00 0.96	0.32 0.28
Bovine Muscle (BCR CRM-184)	Cd	0.013	110	0.65	0.74	0.56	0.45	30	13	36	0.9	2.1	1.00 0.99	0.47 0.38
	Pb[f]	0.24	360	0.55	0.60	0.77	0.51	41	9.7	68	1.7	2.9	1.00 0.99	0.71 0.56
Herring-Gull Egg (GSB-RM)	Cu	2.0[h]	121	1.3	0.96	0.35	0.12	14	98	7.5	0.19	0.98	1.00 1.00	0.19 0.12
Cod Muscle (BCR CRM-422)	Cd	0.016	144	0.6	0.77	0.57	0.39	28	14	31	0.78	2.0	1.00 0.99	0.41 0.36
			144	1.1	0.77	0.37	0.65	31	15	39	0.99	2.2	1.00 1.08	0.36 0.30
	Pb[g]	0.08	116	0.89	0.81	0.44	0.43	27	19	29	0.73	1.9	1.00 1.00	0.42 0.29

[a] certified or reference value; [b] calculated from Eq. [8] and Eq. [14]; [c] identical with sampling constant K_s; [d] mean content and standard deviation calculated from n measurements; [e] analyte content and sampling error calculated with the nugget model from Eq. [6] and Eq. [7]; [f] published in [4]; [g] published in [25]; [h] indicative value

high number of smaller nuggets ($z > 9$) mainly give a contribution to the shape of the basic fraction.

This consideration explains why only a moderate broadening effect by the nugget size distribution must be taken into account when evaluating the nugget fractions.

The determination of z and c_n is rather artificial and does not reflect the real physical properties of the sample powder. Ingamells pointed out, that the development of the sampling theory depends on the proposition: "The sampling characteristics of most mixtures, in which a single element X is of interest, may be duplicated by a hypothetical mixture of uniform grain size which contains only two minerals, each of different X-content" [21].

Despite this limitation of the nugget model, it is evident that the sampling error is determined by the fraction of the largest nugget particles, which come out with the method applied. The calculation of the homogeneity constant, therefore can be assumed to be very reliable.

6 Conclusion

The existence of nuggets in ground mineralogical and geological samples is to be expected and can be explained by the geochemical composition [22]. Nuggets in the samples which are under investigation in this paper are postulated only by statistical evaluation of analytical data. The particles with extreme analyte content sometimes have an unexpected or surprising origin.

For the bovine muscle material, Lücker et al. have shown the endogenous source of the nugget material [23]. The calcificated capsules of dead cysticercus bovis (larval stage of the "ox tapeworm") accumulate lead up to a 500 times higher content compared to the surrounding muscle tissue. These are ideal nugget-forming conditions; a very small mass fraction contains a large analyte portion.

The exogenous origin of nuggets in spruce needles is explained by Wyttenbach et al. [24]. The inclusion of particles from anthropogenic aerosols leads to a significant increase of the material content. Although it was shown only for some elements that are detectable with neutron activation analysis (e.g. aluminium), lead would also have a similarly considerable effect (external > endogenous).

The origin of the nugget effect in the cod muscle can only be supposed. Although care was taken that only the filet material was collected, it is possible, that remains of bones – from which a higher lead and cadmium content is known – are included in the reference material.

The importance of the analytical and statistical tools described in this paper for the certification of biological and environmental CRMs is evident:

If possible, the presence of *small* particle fractions with a very high analyte content must be avoided during CRM sample preparation and production. If present, they must be accurately assessed during the preliminary homogeneity study.

The uncertainty of the certified content given in the certification report, gives information mainly about the degree of mixing, segregation effects ("between-bottle" homogeneity) and the random and systematic errors of the methods used for certification. In addition, the certificate of a CRM should include information about the sampling error to be

expected, if a certain subsample mass is used (micro- or "in-bottle" homogeneity) [3], or in order to evaluate the minimum representative sample size of the CRM correctly [25].

For the determination of the homogeneity constants of a CRM, which can give the user this information, SS-ZAAS proves to be highly suitable. Because of the small sample amount heterogeneity effects clearly emerge. Random analytical errors are known or determinable, so that the degree of homogeneity can be calculated from a variance analysis of the analytical data.

It seems to be impossible to determine and to describe exactly the physical parameters of real biological samples, the evaluation of the solid sampling data by the method presented there gave the most detailed look yet into the distribution of trace elements in real powders of biological origin.

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