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Interlaboratory study on Cu, Pb and Zn determination in soil by inductively coupled plasma optical emission spectrometry using the Bland and Altman test

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The application of the Bland-Altman plot for an interlaboratory study on Cu, Pb and Zn determination over a large concentration range by Inductively Coupled Plasma Optical Emission Spectrometry is described. The method-performance study was selected to estimate the figures of merit within and between two laboratories following the same digestion procedure and analysis technique for 89 soil samples. The regression analysis, the confidence interval of differences mean between results and its 1.96 s limits of agreement revealed a good between-laboratory agreement for a 95 % confidence level. The confidence interval of bias (mg kg^{-1}) was -6±10 Cu, 11±11 Pb and -8 ±10 Zn. The within-laboratory standard deviations (mg kg^{-1}) were similar (29-32; 29-33), while the between-laboratory standard deviations were 47; 51 and 46 mg kg^{-1} for Cu, Pb and Zn up to 1000 mg kg^{-1}. The within-laboratory accuracy and precision were assessed using 4 certified reference materials.

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1. Introduction

Improvement of the quality of chemical measurements and assessment of their validity in accordance with ISO/IEC 17025:2005 Standard represent prominent targets of the Co-operation for Analytical Chemistry in Europe (EURACHEM) as an answer to ever higher demands of regulatory authorities and clients. Approximately 50% of the total measurements performed in the world are chemical measurements characterising a large variety of products, including life security, health and environment. Considering also the trend of economic globalisation, the issue of confidence and approval of measurement results including the testing reports becomes more pregnant. The principal factors involved in the achievement of this goal are standardisation, accreditation and mutual recognise. In this context, the validation of results in laboratories is the aim of many scientific papers, which present the most modern approaches in the matter (Miller, 2000; Massart, 1997; Gunzler, 1994; Harte, 2004; Kurfurst, 2004; Kandler, 2004; Stacey, 2006; Satynarayanan, 2006).

The new Programme of Association of Official Analytical Chemists (AOAC-Programme) aiming at a quicker approval of modern analytical methods mentions the following figures of merit to be determined at their validation: accuracy, recovery, linearity of calibration curve, limit of detection and determination, precision, repeatability and reproducibility, sensitivity and specificity. Quality control (QC) procedures of analysis are related with the quality assurance of samples or lots of particular samples and include: analysis of certified reference materials (CRMs), contaminated samples, duplicates and blanks as well as quality control charts.

Within interlaboratory studies several laboratories analyse the same material in order to distinguish the quality of analytical techniques in the framework of standardisation or during the preparation process of CRMs.

Three types of studies can be distinguished (Massart, 1997; Gunzler, 1994): (i) method-performance or collaborative studies in which the performance characteristics of a specific method are assessed; (ii) laboratory-performance or proficiency studies, in which a material is analysed in which the true concentrations are known or have been assigned in some way, often from the interlaboratory study itself; (iii) material-certification studies in which a group of selected laboratories analyses, usually with different methods, a material to determine the most probable value of the concentration of a certain analyte with the smallest uncertainty possible.

The statistical analysis of experimental data according to Bland and Altman test has been used for years in clinical chemistry and medicine where measurements are usually grouped with few extremes, which can not be excluded in an accurate evaluation of results found within a laboratory (Bland, 1986; Bland, 1999; Lyon, 1991; Grenier, 2000; Dewitte, 2002; Stockl, 2004; Cheng, 2007). The test was also used for statistical evaluation of a particular radiofrequency capacitively coupled plasma optical emission spectrometer (r.f.CCP-OES) for the analysis of biological samples, fresh water gills and fish fillet (Frentiu, 2003), cadmium determination in airborne particulate matter (Frentiu, 2000) in comparison with flame atomic absorption spectrometry (FAAS) and for the validation of the Tessier scheme for speciation of metals in soil (Frentiu, 2007, a).

Compared to the linear regression, which requires an even spread of the data, the Bland and Altman approach allows a statistical evaluation also when results are concentrated in groups on several domains. Beside this,

the Bland and Altman analysis exhibits several advantages such as: (i) it is a simple, uncomplicated statistical approach based on well established operations at hand of chemists; (ii) the plot of the test offers a suggestive evaluation of the agreement between two sets of results found using two methods or in two laboratories; (iii) can be applied both for the normal and abnormal distribution, even if the method providing abnormal distributed results is less reliable.

It was the aim of this paper to implement the Bland and Altman test within an interlaboratory study regarding the determination of several heavy metals in soil over a large concentration range. Thus, the method-performance study was selected in order to estimate characteristics such as within- and between-laboratory precision, systematic error, sensitivity, true limits of detection and determination for Cu, Pb and Zn in soil by inductively coupled plasma optical emission spectrometry (ICP-OES). Systematic errors within- and between laboratory as a result of sample digestion were controlled by analyzing two soils and two water sediments certified materials.

2. Experimental

2.1 Reagents and chemicals

Flucka chemicals had the following qualities: copper, lead, zinc puriss p.a., 32 % (*m/m*) HCl and 65 % (*m/m*) HNO_3 both puriss p.a. Argon from Gaz SRL Cluj-Napoca, Romania (5.0 quality) was used as plasma support gas.

Stock solutions of 1000 μg mL^{-1} Cu, Pb, and Zn were prepared by dissolution of 1.0000 g of high-purity metals in 10 mL of 1+1 HNO_3 (Cu, Pb) or 25 mL of 1+4 HCl (Zn), then diluted to 1 L with 2 % (*v/v*) HNO_3. All required working standards were prepared by dilution of the stock solutions with 2 % (*v/v*) HNO_3.

2.2 Samples collection and digestion procedure

Soils were collected in 2005 from different sites of Baia-Mare, a highly polluted area in North-West Romania (Cordos, 2003; Cordos, 2006; Frentiu, 2007, b). Samples were taken from 5 cm depth using a small shovel, after previous removal of leaves, roots and stones. Soils were sieved through a 2 mm sieve in order to eliminate stones and other materials extraneous to soil, then stored in polyethylene bags for transport to the laboratory. The sieved soils were spread over a polyethylene sheet and air-dried at room temperature for one week turning and breaking the lumps from time to time with a wooden roller to accelerate the drying process. The residual moisture was removed by heating at 105 ± 5^0 C for 3 h. Samples were ground to a fine powder in a tungsten-carbide swing mill for 3 min and sieved through 90 μm sieve. The fraction below 90 μm was further homogenised by mixing in a PVC drum for 1 h, then stored in brown glass bottles at room temperature until analysis. The amount of each sample was divided in two portions and transmitted to the two laboratories to be digested and analysed.

Three parallel samples from each soil were digested using the Lunge mixture as follows. An amount of 2.5000 g from each soil sample prepared as above was exactly weighed into a reaction vessel and turned to slurry with 0.5 – 1 mL of water. Then, 7 mL of 32% HCl followed by 21 mL of 65% HNO_3 were added drop by drop to reduce foaming. A volume of 15 mL of 0.5 mol L^{-1} HNO_3 was added into the absorption vessel connected to the reflux condenser, then the sample was allowed to stand for 16 h at room temperature for slow oxidation of the organic matter of the soil. The temperature of the reaction mixture was slowly raised until reflux conditions were reached and maintained for 2 h. After cooling to room temperature, the content of the absorption vessel was added through the condenser tube and rinsed both with 10 mL of 0.5 mol L^{-1} HNO_3. The extract was centrifuged for 15 min at 6000 rpm to remove the insoluble fraction. The supernatant was diluted up to 100 mL with 2% (*v/v*) HNO_3, then filtrated. Heavy metals were quantitated in the extract prepared as above. The certified reference materials were subject to the same digestion procedure. A blank containing the major elements in soil (1000 Al, 500 Ca, 600 Fe, 150 Mg, 250 K, 50 Na expressed in μg mL^{-1}) and 28 mL Lunge mixture for 100 mL solution was prepared.

2.3 Instrumentation

The determinations were carried out in the Environmental Analysis Laboratory of the Research Institute for Analytical Instrumentation, Cluj-Napoca, Romania (Lab X) using the ICP scanning spectrometer SPECTROFLAME (Spectro Analytical Instruments Kleve, Germany) and in the Regional Laboratory for Complex Spectral Analysis, Babes-Bolyai University Cluj-Napoca, Romania (Lab Y) using the ICP simultaneous spectrometer SPECTRO CIROS^{CCD} (Spectro Analytical Instruments Kleve, Germany). Details about instrumentation and operating conditions are given in Table 1.

All relevant ICP spectrometer operating parameters such as r.f. power level, gas flows, sample uptake rate and positioning of the torch in the front of the optical plasma interface are controlled by the software. Thus, optimum operating conditions can be easily selected.

2.4 Statistics and validation of the results

In order to characterise the determination of Cu, Pb and Zn in soil over a wide concentration range (three orders of magnitude) the method-performance study was selected. The two laboratories followed the same digestion procedure and technique to quantitate the analytes in identical samples to estimate characteristics such as within- and between-laboratory precision, systematic error, sensitivity, limit of detection and determination. The within- and between-laboratory accuracy and precision by ICP-OES were assessed by analysing 4 certified reference materials (LGC 6135 Soil-Hackney Brick Works, LGC Teddington, Middlesex, UK; CRM 025-050 soil, RTC Laramie WY USA; River sediment NCS DC 78301, CNACIS Beijing, China and Freshwater sediment BCR 701 IRMM Geel, Belgium.

Table 1. Instrumentation and operating conditions for the ICP optical emission spectrometers.

Equipment	SPECTROFLAME	SPECTRO CIROS^{CCD}
Generator:	Free – running 27.12 MHz, operated in this experiment at 1200 W	Free – running 27.12 MHz operated in this experiment at 1400 W.
Plasma torch	Inductively coupled plasma, radial viewing observation height: 15 mm Argon flow rates: Outer gas 12 L min^{-1}. Intermediate gas 0.6 L min^{-1}. Nebulizer gas 1 L min^{-1}.	Inductively coupled plasma, axial viewing; torch position (mm): X= -3.9; Y=+3.6; Z=+2.6. Argon flow rates: Outer gas 12 L min^{-1}. Intermediate gas 0.6 L min^{-1}. Nebulizer gas 1 L min^{-1}.
Sample introduction system	2 channel peristaltic pump, glass concentric nebulizer Meinhardt type K (TR-30-K3), double pass Scott type spray chamber sample uptake rate: 2 mL min^{-1} flushing time: 40 s delay time: 20 s	4 channel peristaltic pump, K2 cross-flow nebulizer, double pass Scott type spray chamber sample uptake rate: 2 mL min^{-1} flushing time: 40 s delay time: 20 s
Optics	160-800 nm double scanning monocrhomator, chamber filled with N_2	160 – 800 nm double-grating Paschen-Runge multichannel spectrometer, chamber filled with Ar
Detector	9781 R photomultiplier tube supplied at 1000 V (Thorn EMI, Ruislip, Middlesex, UK).	22 CCD detectors
Data processing	Smart Analyzer Software Background correction: linear two points model, integration time 10 s and 3 successive measurements for each parallel sample	Smart Analyzer Software Background correction: linear two points model (Cu, Zn) and square two points model (Pb), best signal to noise ratio strategy, integration time 45 s and 3 successive measurements for each parallel sample

In order to correct the non-spectral interference of the major elements (Al, Ca, Fe, Mg, K and Na) in the soil, the standard addition method was used for the determination of Cu, Pb and Zn. To evaluate the appropriate amounts to be added the semi-quantitative determination of the analytes (3 seconds integration time) was carried out using SPECTRO CIROS^{CCD}. This enabled also the evaluation of the average matrix of the soil samples.

In the statistical evaluation of the two sets of data obtained in the two laboratories (Lab X, Lab Y) the regression analysis and the Bland and Altman test as well as the one-tailed *F* test to compare the standard deviations of the determinations were used.

In the regression analysis, agreement between the two sets of results is usually considered satisfactory if the correlation coefficient (*r*) and the slope (*b*) of the regression equation do not differ significantly from the unity and the intercept does not differ significantly from zero for a 95 % probability level considered within this study.

The Bland and Altman analysis for the two sets of data from repeated measurements involves the following calculations: *(i)* the mean of repeated measurements on each sample in each laboratory and the within-sample standard deviation (s_{i_X}, s_{i_Y}); *(ii)* the within-laboratory standard deviation:

$$s_{WX} = \sqrt{\frac{\sum s_{i_x}^2}{n}} \quad \text{Eq.(1)} \qquad s_{WY} = \sqrt{\frac{\sum s_{i_y}^2}{n}} \quad (2)$$

where n is the sample size; *(iii)* the differences between means for each sample found in the two laboratories (Δ_i), then the bias estimated by their mean difference ($\bar{\Delta}$) and the standard deviation of differences (s_Δ); *(iv)* the between-laboratory standard deviation ($s_{B_{X-Y}}$) for m_{Xi} and m_{Yi} observations in Lab X and Lab Y on a particular sample:

$$s_{B_{X-Y}} = \sqrt{s_\Delta^2 + \left[1 - \frac{1}{n}\left(\sum \frac{1}{m_{Xi}}\right)\right] s_{WX}^2 + \left[1 - \frac{1}{n}\left(\sum \frac{1}{m_{Yi}}\right)\right] s_{WY}^2} \quad (3)$$

for common cases, when m measurements are performed on each sample in each laboratory, the Eq.(3) becomes:

$$s_{B_{X-Y}} = \sqrt{s_\Delta^2 + \left(1 - \frac{1}{m}\right) s_{WX}^2 + \left(1 - \frac{1}{m}\right) s_{WY}^2} \quad (4)$$

(v) the limits of agreement of the results $\bar{\Delta} \pm 1.96 s_{B_{X-Y}}$ and the confidence interval of $\bar{\Delta}$ ($\bar{\Delta} \pm t \frac{s_{B_{X-Y}}}{\sqrt{n}}$), for a 95 % confidence level, where *n* is the sample size and *t* the Student coefficient for (*n-1*) freedom degrees; *(vi)* the 95% confidence interval for limits of agreement:

$$\bar{\Delta} \pm 1.96 s_{B_{X-Y}} \pm t \cdot s_{LL,UL} \quad (5)$$

where $S_{LL,\ UL}$ is the standard deviation of lower and upper limit of agreement

$$s_{LL,UL} = \sqrt{\frac{s^2_{B_{X-Y}}}{n} + \frac{1.96^2}{2s^2_{B_{X-Y}}}\left(\frac{s^4_{\Delta}}{n-1} + \frac{(m-1)s^4_{WX}}{nm^2} + \frac{(m-1)s^4_{WY}}{nm^2}\right)} \quad (6)$$

(vii) plotting the differences between means found in each laboratory (Δ_i) against the mean values; the bias, limits of agreement and their confidence interval for 95% confidence level are labelled.

According to the test described by Bland and Altman, there is no significant bias between the two sets of results if the confidence interval of the mean difference ($\bar{\Delta}$) contains the zero value and the differences for each sample between laboratories (Δ_i) lie between the limits of agreement of the results. Also, the confidence interval for the limits of agreement should be reasonable narrow, which is the case for a large sample size.

3. Results and discussion

3.1 Line selection and detection limits

For each spectrometric system the most sensitive lines, free from spectral interference, were selected. Thus, for SPECTROFLAME the selected wavelengths (nm) were: Cu(I) 324.754, Pb(I) 283.307 and Zn(I) 213.856, while for SPECTRO CIROS^{CCD} Cu(I) 324.754, Pb(II) 220.351 and Zn(I) 213.856.

The true limits of detection for Cu, Pb and Zn in soil for the spectrometric systems in the two laboratories were estimated based on the 3σ criteria using the parameters of the calibration plots in the presence of (μg mL^{-1}) 1000 Al, 500 Ca, 600 Fe, 150 Mg, 250 K, 50 Na (Table 2).

These concentrations matched both with the average matrix composition of soils as revealed by the semi-quantitative analysis and the certified materials. All calibration solutions contained also 28 mL Lunge mixture for 100 mL solution. The limit of determination was considered as 5 times the limit of detection.

Table 2. Line selection and true detection limits (3σ) for Cu, Pb and Zn in soil for the two spectrometric systems

Element /line /nm	Calibration range/ μg mL^{-1}	Limit of detection /mg kg^{-1} dry mass*	
		Lab X	Lab Y
Cu (I) 324.754	0-50	0.4	1
Zn(I) 213.856	0-50	0.3	1
Pb(I) 283.307	0-50	1.5	-
Pb(II) 220.351	0-50	-	5

* calculated according to the dry mass (2.5 g of the sample dissolved in 28 mL Lunge mixture and filled up to a final volume of 100 mL) and interelement effect of: 1000 Al, 500 Ca, 600 Fe, 150 Mg, 250 K, 50 Na all expressed in μg mL^{-1}

The excellent sensitivity of the two spectrometric systems enables the quantitation of Cu, Pb and Zn in soil at levels much below the limiting values (60 Cu; 200 Zn and 100 Pb mg kg^{-1}, respectively). Thus, the SPECTROFLAME allows the determination of amounts (mg kg^{-1}) of at least 2 Cu, 1.5 Zn and 7.5 Pb, while SPECTRO CIROS^{CCD} 5 Cu, 5 Zn and 25 Pb, both with a relative standard deviation (RSD) of below 10%. The higher detection and quantitation limits for SPECTRO CIROS^{CCD} are due to the lower sensitivity of the CCD detectors as compared to the photomultiplier tube. On the other hand, the simultaneous character of the SPECTRO CIROS^{CCD} allows a full spectrum to be measured in 3 s, offers a high speed sample throughput and results in cost decrease per analysis compared to the scanning SPECTROFLAME.

3.2 CRMs analysis. Accuracy and precision within-and between-laboratory

The found and certified results for four standard reference materials are compared in Table 3.

In both laboratories, there was a good agreement between leachable contents extracted in Lunge mixture and the certified values of Cu, Pb and Zn regarding the accuracy and precision. The recovery degrees (%) of the certified mean for Cu, Pb and Zn calc ulated using the average found values and standard deviation were 101±10, 99±10 and 100±6 in Lab X and 102±10, 95±10 and 99±7 respectively, in Lab Y. The general relative standard deviations (%) for Cu, Pb and Zn were 9.3; 10.4 and 5.6 in Lab X and 9.6; 10.8 and 6.7 in Lab Y, respectively.

3.3 Statistical comparison of results between-laboratories

Contents and standard deviations for Cu, Pb and Zn (3 parallel samples) in 89 soil samples found in the Environmental Analysis Laboratory of the Research Institute for Analytical Instrumentation (Lab X) and in the Regional Laboratory for Complex Spectral Analysis (Lab Y) are presented in Table 4.

Linear regressions for Cu, Pb and Zn are illustrated in Fig. 1a-3a, while the corresponding Bland and Altman plots are shown in Fig. 1b-3b. For 3 parallel samples of each soil and a 95% confidence level the parameters of the regression plots and the data calculated for the Bland and Altman test are presented in Table V. According to the parameters of the regression plots and Bland and Altman tests there is a good agreement between the two laboratories for a 95 % confidence level.

For all three elements, the confidence intervals of the intercept include the zero value and the slopes are not significantly different from unity. The within-laboratory standard deviations are similar (29-32 and 29-33 mg kg^{-1}) in accordance with the signal reproducibility in ICP-OES. The pooled standard deviations in the two laboratories characterise the analysis of the 3 metals over a large range of concentration in soil up to 1000 mg kg^{-1}.

Table 3. Comparison of the found leachable Lunge content of Cu, Pb and Zn (m=5 parallel samples) in the two laboratories using ICP-OES and the certified concentrations in the reference materials.

Certified material	Element	Certified value ±1s/ mg kg^{-1}	Found value±1s/ mg kg^{-1}	
			Lab X	Lab Y
LGC 6135 soil	Cu	105±5	103±8	104±5
	Pb	391±16	373±32	355±32
	Zn	316±41	318±18	321±27
CRM 025 050 soil	Cu	7.76±1.68	8.65±1.34	8.11±1.27
	Pb	1447±203	1370±226	1352±237
	Zn	51.8±8.29	52.1±4.11	48.6±3.88
NCSDC 78301 sediment	Cu	53±3	51±3	54±4
	Pb	79±6	81±5	78±4
	Zn*	251	255±12	252±14
BCR 701 sediment	Cu*	275±13	273±10	279±19
	Pb*	143±6	148±10	140±10
	Zn*	454±19	445±12	458±18

* Indicative values of the supplier

Table 4. Analytical results in mg kg^{-1} dry mass for Cu, Pb and Zn in soil in the two Labs (m=3).

Sample	Lab X mean±1s/ mg kg^{-1}			Lab Y mean±1s/ mg kg^{-1}		
	Cu	Pb	Zn	Cu	Pb	Zn
1.	750±62	874±42	920±39	811±45	810±45	960±45
2.	665±38	760±35	490±24	623±32	725±40	475±30
3.	586±56	770±44	570±26	536±60	717±32	540±30
4.	559±34	485±26	370±40	510±54	510±39	416±37
5.	831±42	314±17	605±26	754±70	280±19	565±15
6.	373±50	790±30	490±19	434±37	764±45	520±24
7.	512±35	262±14	950±39	495±35	276±14	900±44
8.	487±44	835±45	885±34	536±65	758±33	845±47
9.	260±24	455±26	980±45	281±27	436±21	920±49
10.	190±13	375±17	710±28	190±14	353±19	630±31
11.	515±25	350±20	470±25	540±35	335±25	420±21
12.	384±24	339±22	545±31	370±60	312±24	590±27
13.	53±3	203±18	325±31	56±4	215±21	290±25
14.	410±47	1000±55	610±38	487±49	910±40	656±33
15.	179±13	845±55	515±22	193±16	805±41	545±29
16.	48±3	335±18	240±18	41±3	305±22	270±15
17.	54±2	729±36	200±16	54±4	745±32	235±10
18.	56±4	256±19	290±15	54±5	237±12	265±19
19.	477±30	225±15	740±38	420±35	204±21	785±40
20.	495±36	665±48	525±30	471±28	620±34	565±35
21.	255±23	865±55	425±29	226±26	880±54	395±24
22.	51±3	458±28	325±34	61±5	435±37	310±32
23.	311±16	880±49	445±26	330±18	815±41	460±23
24.	652±30	167±9	630±28	641±49	175±8	655±25
25.	557±28	441±26	589±34	570±44	457±32	623±27
26.	190±13	860±36	294±18	184±18	823±36	278±7
27.	159±13	343±27	259±18	149±16	320±29	275±16
28.	196±23	287±26	690±35	216±25	309±22	729±44
29.	131±24	338±24	720±41	151±28	354±30	755±32
30.	696±40	310±28	634±33	630±40	344±22	656±43
31.	661±38	570±36	700±37	715±32	595±36	730±33
32.	387±25	629±36	690±28	382±28	644±44	675±32

Sample	Lab X mean±1s/ mg kg^{-1}			Lab Y mean±1s/ mg kg^{-1}		
	Cu	Pb	Zn	Cu	Pb	Zn
33.	932±95	625±36	630±32	982±84	630±30	670±40
34.	82±13	210±13	494±38	74±8	224±18	419±33
35.	580±47	620±24	448±26	636±39	639±32	465±26
36.	68±6	189±16	360±26	89±11	178±12	376±26
37.	123±12	368±24	492±32	118±22	372±24	522±42
38.	83±9	183±25	252±27	95±7	195±22	290±19
39.	98±10	206±25	208±15	90±8	187±20	244±15
40.	171±23	357±27	807±45	165±28	367±32	835±42
41.	175±14	905±48	377±24	162±14	885±58	394±19
42.	207±30	316±21	457±29	260±30	326±30	473±25
43.	277±30	745±44	515±33	289±30	772±48	530±27
44.	228±30	507±24	466±29	235±40	485±39	422±24
45.	164±18	595±50	818±36	177±21	560±40	850±47
46.	85±4	185±13	295±16	78±4	175±13	277±16
47.	80±3	293±28	276±15	87±5	260±24	265±15
48.	287±26	943±54	802±48	316±20	966±62	781±48
49.	53±4	160±20	244±17	47±4	137±16	228±11
50.	306±36	545±35	804±48	291±24	555±30	887±38
51.	143±22	480±24	671±35	139±18	448±24	657±42
52.	105±24	492±36	415±46	127±26	517±48	480±36
53.	383±53	545±28	652±30	416±55	490±38	673±42
54.	465±29	803±47	648±37	486±40	830±67	670±41
55.	411±29	895±44	499±25	453±40	834±55	517±28
56.	227±25	332±25	750±36	252±30	326±33	724±40
57.	262±25	282±15	606±34	269±25	254±22	625±25
58.	340±36	190±34	631±29	351±25	150±34	689±27
59.	208±27	197±13	300±21	231±27	174±13	326±28
60.	128±19	227±30	294±24	147±18	256±35	275±30
61.	264±19	187±21	241±27	247±25	216±28	257±22
62.	142±14	293±30	279±23	130±14	327±20	297±18
63.	183±15	220±20	211±20	160±14	206±15	228±12
64.	438±38	384±33	304±26	463±29	333±37	326±22
65.	167±16	290±25	429±27	189±11	257±17	415±38
66.	131±7	209±25	478±30	144±12	245±29	449±23
67.	218±22	323±31	295±28	228±14	312±44	283±16
68.	188±15	335±26	211±16	215±15	358±18	225±16
69.	49±3	259±14	255±26	44±4	221±12	238±15
70.	52±4	260±14	250±27	72±3	272±16	270±13
71.	179±13	192±15	356±33	191±18	210±15	386±25
72.	172±13	209±16	310±18	185±14	200±15	287±24
73.	226±15	350±27	368±16	215±17	321±24	347±24
74.	381±36	293±27	277±19	412±30	304±22	260±29
75.	425±45	320±28	268±15	400±40	295±25	280±19
76.	433±43	910±60	316±34	452±37	1000±80	301±34
77.	411±43	1000±60	228±21	446±37	925±55	237±21
78.	420±36	470±25	266±15	455±28	405±27	253±15
79.	566±50	368±16	410±18	626±50	323±28	435±23
80.	209±15	334±33	450±19	226±15	318±25	480±33
81.	44±6	170±15	230±25	38±5	127±20	255±24
82.	45±3	365±24	260±16	46±4	329±18	245±12
83.	635±28	960±58	857±27	629±27	925±44	878±31
84.	525±39	696±35	683±24	501±24	718±30	653±17

Sample	Lab X mean±1s/ mg kg^{-1}			Lab Y mean±1s/ mg kg^{-1}		
	Cu	Pb	Zn	Cu	Pb	Zn
85.	567±42	615±34	676±38	624±60	700±50	700±35
86.	940±55	635±24	905±40	877±60	659±26	935±36
87.	294±16	840±48	236±17	288±18	783±34	227±12
88.	278±15	545±26	350±25	270±26	570±28	380±27
89.	78±6	187±17	275±18	75±7	170±20	280±14

Table 5. Linear regression parameters and data calculated for the Bland and Altman test for a 95% confidence level and 89 soil samples (m=3 parallel samples).

	Cu	Pb	Zn
Regression analysis			
intercept	9.07±10.92	1.89±14.98	5.90±16.88
slope	0.991±0.028	0.973±0.028	1.000±0.032
correlation coefficient	0.991	0.991	0.989
Bland and Altman results			
within-laboratory standard deviation/ mg kg^{-1}			
Lab X (s_{wx})	30	32	29
Lab Y (s_{wy})	32	33	29
between-laboratory standard deviation, (s_{Bxy})/ mg kg^{-1}	47	51	46
confidence interval of bias ($\overline{\Delta} \pm t \frac{s_{B_{X-Y}}}{\sqrt{n}}$)/ mg kg^{-1}	-6±10	11±11	-8±10
confidence intervals for limits of agreement ($\overline{\Delta} \pm 1.96 s_{B_{X-Y}} \pm t \cdot s_{LL,UL}$)/ mg kg^{-1}	-98±14 86±14	-88±14 110±14	-98±13 82±13

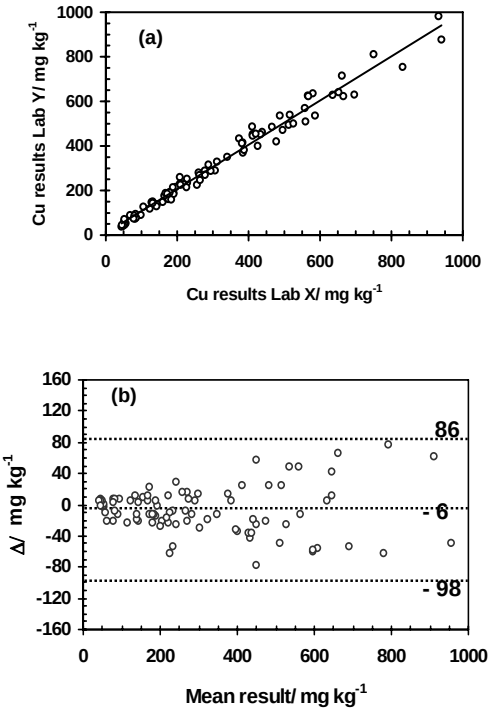

Fig. 1. (a) Copper regression plot and (b) Bland and Altman plot (89 soil samples).

On this concentration interval the between-laboratory standard deviations (mg kg^{-1}) are: 47 (Cu); 51 (Pb) and 46 (Zn). In the case of Cu, for samples with less than 60 mg kg^{-1}, the within-laboratory standard deviations are significantly lower (4 mg kg^{-1} in Lab X and 5 mg kg^{-1} in Lab Y), while the between-laboratory standard deviation is of only 8 mg kg^{-1} compared to 47 mg kg^{-1} for the whole concentration range. According to the previous discussion and Fig.1b-3b the standard deviation depends on the element concentration in soil.

The low negative bias (mg kg-1) for Cu (-6) and Zn (-8) towards the Lab Y and low positive bias for Pb (+11) towards the Lab X are unimportant for the work envisaged as in the analysed soils the concentration ranges of these elements are very large. Considering the 95% confidence interval, the bias between laboratories is random and the null hypothesis is retained even for Pb, which is at limit. This could be attributed to the different strategies necessary to correct background for the two instruments in this case. Also, the limits of agreement of the differences between laboratories include all 89 samples in the case of Cu and Zn, but one in the case of Pb. The confidence intervals of the limits of agreement are reasonable narrow, however they include the extreme value of Pb.

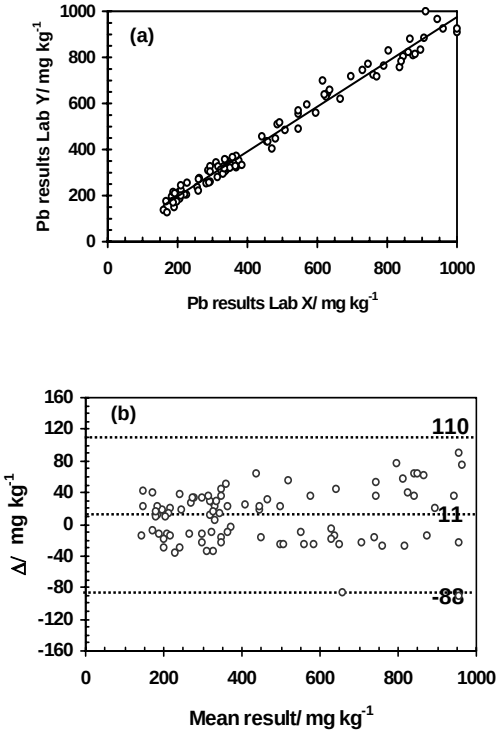

Fig. 2. (a) Lead regression plot and (b) Bland and Altman plot (89 soil samples).

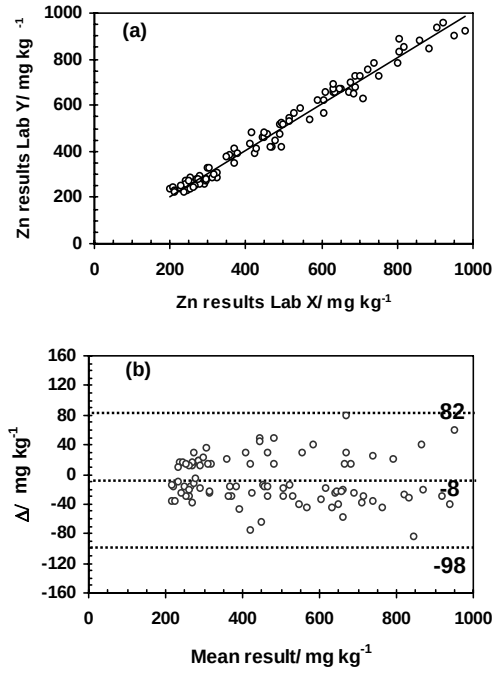

Fig. 3. (a) Zinc regression plot and (b) Bland and Altman plot (89 soil samples).

4. Conclusions

An agreement study between two laboratories using the method-performance strategy was conducted in the determination of Cu, Pb and Zn in soil samples based on regression analysis and Bland and Altman test, respectively. The Bland and Altman test is very suggestive, simple both to do and to interpret by chemists. Generally, the plots are useful for the presentation and interpretation of the method-comparison studies. The test could be successfully applied to results obtained in fields other than the clinical one, such as analytical chemistry for environmental applications, where both normal and extremely high contents of pollutants are encountered. It has been found that extremely attention must be paid both to the preliminary sample preparation and digestion protocol in order to get a good agreement between results.

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