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QUANTIFYING UNCERTAINTY IN ANALYTICAL MEASUREMENTS

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ABSTRACT

In providing chemical, biochemical and agricultural materials testing services for quality specification, the analytical chemists are increasingly required to address the fundamental issues related to the modern concepts of Chemical Metrology such as *Method Validation, Traceability and Uncertainty of Measurements.* Without this knowledge, the results cannot be recognized as a scientific fact with defined level of acceptability. According to ISO/IEC 17025:2005, this is an essential requirement of all testing laboratories to attain competence to test materials for the desired purpose. Of these three concepts of chemical metrology, the most complex is the calculation of uncertainties from different sources associated with a single measurement and incorporate them into the final result(s) as the expanded uncertainty(U_E) with a defined level of reliability (e.g., at 95% CL). In this paper the concepts and practice of uncertainty calculation in analytical measurements are introduced by using the principles of statistics. The calculation procedure indentifies the primary sources of uncertainties and quantifies their respective contributions to the total uncertainty of the final results. The calculations are performed by using experimental data of **Lead (Pb) analysis in soil** by GF-AAS and *pesticides analysis in wastewater* by GC-MS method. The final result of the analytical measurement is expressed as: **Result (mg/kg) = Measured Value of Analyte (mg/kg) ± Uncertainty (mg/kg),** where the uncertainty is the parametric value associated with individual steps in measurements such as sample weighing**($\mathbf{U_m}$),** extraction of analyte (**$\mathbf{U_e}$**) (Pb from soil or pesticides from water), volumetry in measurement (**$\mathbf{U_v}$**), concentration calibration(**$\mathbf{U_x}$**), etc. The propagation of these individual uncertainties from different sources is expressed as combined relative uncertainty (U_c), which is calculated by using the formula:

$$\text{Combined uncertainty } \left(\frac{U_c}{c}\right) = \sqrt{\left(\frac{Ux}{x}\right)^2 + \left(\frac{Um}{m}\right)^2 + \left(\frac{Uv}{v}\right)^2 + \left(\frac{Ue}{e}\right)^2 + \ldots}$$

The overall uncertainty associated with the final result of the analyte is expressed as *Expanded Uncertainty (U_E)* at certain level of confidence (e.g. 95%). The *Expanded Uncertainty* is calculated by multiplication of *Combined Uncertainty (U_c)* with a coverage factor (K) according to the proposition of level of confidence. In general, the level of confidence for enormous data is considered at 95%, CL where K is 2. Hence, the final result of the analyte is expressed as: X ± U_E (unit) at 95% CL, where $U_E = 2U_c$.

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Key words: Measurement uncertainty, atomic absorption spectrophotometry, GC-MS, heavy metals, pesticides, soil, wastewater

INTRODUCTION

Chemical and biochemical analyses of materials for quality specifications are a continuous need of a modern society. The role of these analyses is very important, for example, in food, drink and air quality analysis for public health assuring, in healthcare services, and in trade and research. In all these cases, what is most important is to get reliable analytical data. And this is achievable when the testing laboratories follow the international standard for assuring the quality of measurement data, namely, ISO/IEC 17025:2005 (General requirements for the competence of testing and calibration laboratories) (Hibbert, B. D., 2007)

This ISO standard has a set of management and technical requirements. The technical requirements of this standard are linked to the science of measurements; meaning that metrological issues such as *traceability, uncertainty and validation* are the essential components of quality assured analytical data reported with defined levels of uncertainties and defensible confidence limits. The scope of this paper is, however, limited to calculation of uncertainty in analytical measurements that can be uniformly applied to all quantitative chemical analyses.

The uncertainty in a measurement is the interval on the measurement scale within which the true value lies with a specified probability, when all sources of error are taken into account. In modern concepts of chemical metrology, analytical chemists are increasingly required, as a standard practice, to make a statement on the level of uncertainty associated with the estimate of a measurand. Without this knowledge, the measurement cannot be properly interpreted and the acceptability of the test results for a specific purpose cannot be ensured (DOI 10.1039/AN 9952002303, 1995, EUROCHEM/CITAC Guide CG 4, 2012). The measurement of uncertainty in quantitative chemical analysis influences the data generated from each involved in the experimental measurements such as *weighing of a sample, extraction of analyte, volumetry of sample solution and calibration of the measuring instrument.* The uncertainty measurement in each of these steps thus becomes the most important unifying principle in the overall data quality assessment (GUM, 2008).

This paper briefly introduces the concepts and practices of data quality evaluation in analytical chemistry and outlines the basic procedures of calculating uncertainty in analytical measurement, using the principles of statistics (Harris, C. D., 2010). In the procedure, the primary sources of uncertainty are identified and their respective contributions to the total uncertainty in the final results are calculated for routine measurements, for example, trace metals analysis in different sample matrices. The approach adapted to the calculation of uncertainty in solid and liquid sample matrices is consistent throughout the data quality evaluation process so that it can be uniformly applied to all quantitative analyses and thus becomes acceptable to the whole analytical community (Harvey, D., 2000).

Concepts of Error and Uncertainty

The traditional concept of error in measurements assumes that there is a single true value of a measurand and it can be reached by performing repeated measurements done with care and attention to details. In reality, a measurement procedure defines, to the extent possible, what is measured. On the other hand, uncertainty can only describe a range within which there is a reasonable chance of finding a value that might be attributed to the measurand. A true value can only be defined as the quantity value consistent with the definition of a quantity, which in other words allows the possibility of finding many true values in a range that is defined as the statistical probability of finding the most probable value within the range (Harvey, D., 2000).

Percent Error in Measurements

Percent error, sometimes referred to as fractional difference, measures the accuracy of a measurement by the difference between a measured or experimental value, E, and a true or accepted value, A. The percent error is calculated from the difference between the two using the following equation:

$$\% \text{ Error} = \frac{E - A}{A} \tag{1}$$

Types of Errors in Measurements

Type B Uncertainty: Systematic error

Type B Uncertainty, also known as systematic error or determinate error, arises from the flaw/defect in the equipment used or in the design of the experiment. In this case, if the experiment is repeated under identical conditions, the error would be reproducible. Systematic errors are always in one direction and are of the same magnitude when repeatedly measured. These errors can be found out and corrected by analyzing a CRM for the same parameter. For example, an incorrectly standardized pH meter with a buffer will produce systematic error. If one uses a buffer with known pH of 7.00 units to calibrate it, it will give result like 7.08, 0.08 units lower. With this pH meter if measured a pH of 5.60, the actual pH would be 5.68 units. This error can be found by using another buffer with certified pH value.

Type A Uncertainty: Random error

Type A Uncertainty, known as random or indeterminate error, arises from a number of uncontrolled variables such as *water quality used in preparation, unstable blank correction, errors in calibration, etc*. Random errors cannot be corrected. In repeated measurements, random errors have equal chance of being positive and negative. Instrument electronic noise is also a source of random error. If you make ***n*** number of measurements, where ***n*** is greater than 30, for example, each data will be different and errors in the measurement will follow a normal distribution like (Kateman, G. and L. Buydens, 1993).

$$f(x) = \frac{1}{\alpha\sqrt{2\pi}} \exp\left[\frac{(x-\text{«})^2}{2\pm_2}\right] \qquad (2)$$

where µ is the mean, x is the individual result, σ is the standard deviation, π has the usual value. This uncertainty combined with other sources will give the measurement uncertainty with certain degree of probability; say for example, 95% (2σ standard deviation), or 99% (3σ standard deviation) (Muller, J. C. and J. N. Muller, 1993).

The estimation of measurement uncertainty is a process designed to increase understanding of the measurement result by assessing the factors that influence the measurement. It is also convenient to treat all experimental data statistically to distinguish between random measurement errors, the standard deviation of which can be estimated from repeated measurements, and systematic measurement errors that are one-way deviation which can be measured or estimated and then corrected or included in uncertainty budget. These concepts are explained here in this paper with support of primary measurement data generated in analytical laboratories (ISO/IEC 17025, 2005).

In the traditional *Error Approach* (or True Value Approach), the objective is to determine an estimate of the true value that is as close as possible to that single true value. The deviation from the true value is composed of random and systematic errors. In the *Uncertainty Approach*, the objective of measurement is not to determine a true value as closely as possible. Rather, it is assumed that the information from measurement only permits assignment of an interval of reasonable values to the measurand, based on the assumption that no mistake has been made in performing the measurement.

Sources of Uncertainties

The Steps involved in any analytical measurement are (i) sample preparation (by dissolving a definite weight of a sample in a definite volume), (ii) preparation of standards solutions using CRM, (iii) calibration of measuring equipment and (iv) preparation of sample blank. Each of these steps has some degrees of uncertainties, depending on the level of **'GLP'** (good laboratory practice) that has been established in the laboratory. How the contribution from each of these sources to the final uncertainty in the result (combined & expanded uncertainty) is calculated, is discussed in this paper with examples of **Pb** analysis in soil and pesticides in wastewater.

MATERIALS & METHODS

Two analytical instrumentations with different sample matrices were considered for this exercise of calculating measurement uncertainty. For this purpose, data were collected from lead (Pb) analysis in soil (as solid sample matrix) by using GF–AAS (Shimadzu, Model: 6800) method and Organophosphorus pesticides analysis in wastewater (as liquid sample matrix) by using GC–MS (Shimadzu, Model: QP 2010) methods. The following laboratory requirements under Good Laboratory Practices (GLP) were taken into consideration for analytical measurements of the selected parameters.

- Validated analytical method and calibrated equipment (Analytical Instrumentations & Balance) are used.
- Certified Reference Materials (CRMs) are used for equipment calibration
- Appropriate method blanks are prepared for blank correction in each measurement.
- Qualified analysts perform the analysis.
- Availability of Class A Glassware be ensured
- Analar Grade reagents and Reagent grade water with **MΩ ≥ 17** were used.
- Clean and safe laboratory environment was ensured.

Data acquisition from Pb Measurement by GF–AAS

The first exercise of analytical problem considered here for ***Quantifying Uncertainties*** is the measurement of Lead (Pb) in a soil sample using Atomic Absorption Spectroscopy (AAS). The following four steps involved in the procedures were considered to be significant sources of uncertainties in this analysis.

(1) 0.5008 g dried soil sample was weighed using an electronic balance. The uncertainty at standard 500 mg was ± 0.25 mg under the repeatability condition of the electronic balance. The standard mass was calibrated from National Metrology Laboratory of Bangladesh Standards and Testing Institution (BSTI).

(2) The soil sample was digested in HNO_3 matrix (Analar Grade from BDH England) and the final volume of digested solution was prepared in a 100 mL volumetric flask. Uncertainty stated by the manufacturer of Class A glassware for the 100 mL volumetric flask as ± 0.05mL (at 20°C). It was considered that the uncertainty regarding dissolution of analyte through digestion procedure is negligible.

(3) Five Lead (Pb) standards were prepared in the concentration range of **0, 5, 10, 15 and 20 µg/L (ppb)** from certified reference material of Sigma Aldrich**.** The instrument (AAS) response was measured in absorbance (Abs) against standard

lead (Pb) solutions and a standard calibration plot was prepared, with triplicate measurements for each standard concentration. The absorbance of digested sample (step-2) was also measured in triplicates with GF–AAS technique and the concentration of Lead (Pb) in the digested solution was calculated from the calibration plot, expressed in µg/L. All the measured data are shown in **Table 1.**

(4) The repeatability of instrumental response or the precision of the test result was evaluated using quality control standard (10 µg/L) prepared from the CRM. The repeatability data are shown in **Table 1.**

Table 1: Absorbance of GF-AAS from standard calibration and sample for Pb Measurement

Pb (µg/L)	Abs (Series – 1)	Abs(Series – 2)	Abs (Series – 3)
Known x_i	y_i *(1)*	y_i *(2)*	y_i *(3)*
0	-0.0004	0.0004	-0.0003
5	0.0955	0.0956	0.0950
10	0.1786	0.1753	0.1704
15	0.2564	0.2550	0.2531
20	0.3333	0.3292	0.3221
x(Digested solution)	**0.1422**	**0.1512**	**0.1447**
CRM 10 «g/L	**0.1733**	**0.1745**	**0.1718**

The final concentration of Pb in soil sample was calculated from the actual soil sample taken (from step-1), expressed in **µg/kg (ppb)**. The uncertainties in the final concentration of Pb in soil have been calculated using the procedures (step 1-4) discussed in the **Results and Discussion** section.

Data acquisition from Measurement of Organophosphorus Pesticides by GC–MS

The second example considered for quantifying uncertainties is the analysis of Organophosphorus Pesticides (OPP) in wastewater samples using Gas Chromatography–Mass Spectrometry (GC–MS). In this excurse a Proficiency Testing (PT) sample from Absolute Standards Inc, USA, containing 15 types of OPP compounds was taken for uncertainty calculation. For concentration calibration of the GC-MS method, a certified reference material (CRM), also from Absolute Standards, having the certified concentration of 1000µg/L was used.

The original PT sample of 1 mL, provided in an ampoule, was diluted to 1000 mL as per the sample preparation guide. The finally evaluated PT results where each analyte was assigned a value from interlaboratory comparison study were used for uncertainty calculation. The typical GC-MS chromatogram for standard OPP is shown in the Figure 1. The uncertainty calculation procedures are discussed in the **Results and Discussion** section.

The uncertainty of test results was calculated for two OPP compounds, **Parathion Methyl and Malathion** out of 15 compounds of OPP in the sample as a model calculation. The sources of uncertainties in the analytical procedure were assumed to be each of the following steps involved in the analytical procedure.

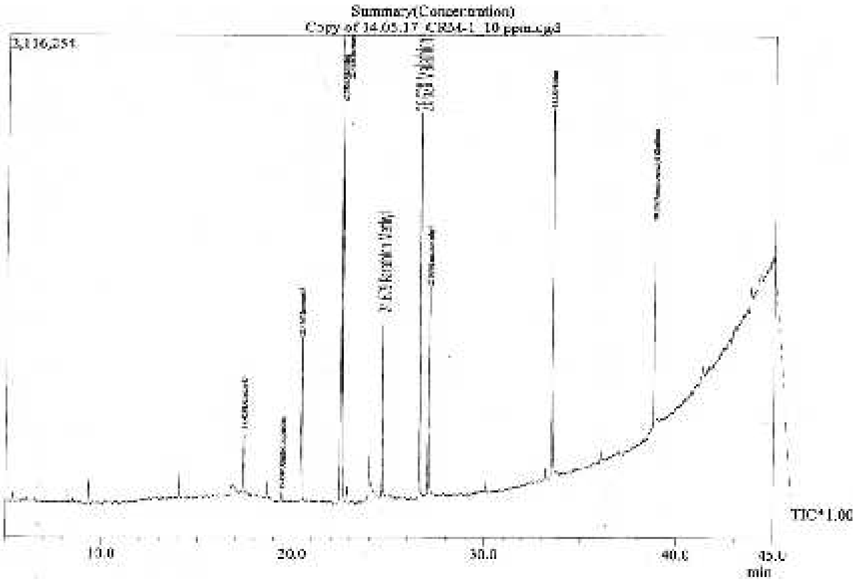

Fig. 1. GC-MS chromatogram of Organophosphorus pesticides (OPP) certified reference material (CRM)

(1) 1 mL supplied sample was pipetted out from the ampoule and diluted to 1000 mL in a volumetric flask, using Reagent Grade water (**MΩ ≥ 17**). The uncertainties from volumetry were ±0.01mL for 1 mL sample and ±0.5mL for 1000 mL sample.

(2) The pesticides in diluted sample were extracted in organic solvent (Hexane, HPLC Grade by RCI Labscan Ltd, Thailand) and recovered through evaporation of solvent in a rotary evaporator. The extraction efficiency of such solvent extraction procedure is generally considered to be 85%.

(3) The final volume of the sample was constituted to 1 mL solvent (mixture of Acetone & Hexane) for injecting into the GC column for separation of different components. The injection volume was 2 μL for each calibration standard, sample and QC standard.

(4) Standard calibrations were carried out for each compound of OPP using the GC-MS instrumentation, where mean peak areas were recorded against standard concentration by the application software of the analytical instrument. Table 2 shows the calibration data for the two compounds of OPP, used for quantifying measurement uncertainty.

Table 2: Mean Peak area from GC-MS Chromatogram against standard Parathion Methyl & Malathion

Parathion Methyl (µg/mL)	Peak Area (Mean)	Malathion(µg/mL)	Peak Area (Mean)
Known x_i	y_i	Known x_i	y_i
2.5	9915	2.5	13693
5	25274	5	36701
10	46199	10	62757
20	147461	20	200099
40	292304	40	360278

(5) The peak area of individual compound of OPP in PT sample was also recorded repeatedly 5 times by the instrument. In addition, 10 ppm QC standard of each compound was also used to see the run to run variation and recovery factor. Table 3 shows the peak areas of two compounds of PT sample and the QC standard. Though the application software showed the concentration of each OPP compounds of PT sample and QC standard, the concentration of two compounds have been calculated here using **Least Squares Method** for the exercise of Measurement Uncertainty.

Table 3: Peak area from GC-MS Chromatogram against unknown Parathion Methyl & Malathion in PT sample

	Parathion Methyl (Peak Area from GC-MS)		Malathion (Peak Area from GC-MS)	
	PT Sample (extract)	QC Standard (10 ppm)	PT Sample (extract)	QC Standard (10 ppm)
	97185	461992	117263	62757
	105872	437634	122193	64229
	121314	402111	135730	57163
	135771	433077	141302	56387
	135170	418537	152160	60830
		439061		60653
		455907		57278
		439784		
Count(n)	5	8	5	7
Mean	119062	436013	133729	59899
SD	17294	19124	14188.6	3028.8
%RSD	14.5%	4.4%	10.6%	5.1%

Computation of Measurement Uncertainty

The statistical method for *linear least squares fit* in concentration calibration is applied for the calculation of uncertainty from the standard calibration that is considered as one of the major sources of uncertainty in the analytical procedure. The statistical equations for linear calibration plot are described in the reference books (Harris, C. D., 2010, Kateman, G. and L. Buydens). These equations are used in this exercise to explain the process involved in measurement uncertainty. Simple tabular format for data acquisition and a scientific calculator can be used for this exercise, as shown in the uncertainty calculation procedure for Pb analysis in soil. Alternatively, personal computer based MS Excel spreadsheet application software can also be used for data acquisition and necessary calculation of measurement uncertainty, as used here for pesticides analysis in wastewater.

RESULTS AND DISCUSSION

Process steps of measurement uncertainty for Lead (Pb) in soil sample

1. Construction of linear calibration plot using Least Squares Method (LSM) and measure unknown concentration

Table 1 shows that three absorbance values (replicate) were measured against each standard concentration of **Lead (Pb).** The calculation procedures of uncertainties associated with lead concentration measurement in a soil sample are described below.

In this regard, the 'Least Squares Equation' (3) is the appropriate one for constructing the calibration curve.

$$y = mx + b \tag{3}$$

where,

y = Abs (instrument response against standard analyte)

x = concentration (µg/L, standard analyte)

m = slope of linear equation

b = intercept on y axis of liner equation

x_i = individual value of standard analyte

y_i = individual value of absorbance against each standard analyte

m & b values are calculated using the following least-squares equations:

$$m = \frac{n\sum(x_i y_i) - \sum x_i \sum y_i}{n\sum(x_i^2) - (\sum x_i)^2} \tag{4}$$

$$b = \frac{\sum(x_i^2)\sum y_i - \sum(x_i y_i)\sum x_i}{n\sum x_i^2) - (\sum x_i)^2} \tag{5}$$

The computation of ***m* &*b*** values using experimental data (x_i &y_i) is shown in Table 4**.** Accordingly, the calculated values of m & b are found to be 0.016 & 0.007 respectively.

Table 4: Variables for calculating slope (m) and y-intercept (b)

Series	Pb (µg/L)	Abs	x_i^2	x_iy_i	**Calculation of m & b value from Eq. 4 & 5**
	x_i	y_i			
Series – 1	0	-0.0004	0	0	The number of data set n is 15 (5 data sets from each series)
	5	0.0955	25	0.4775	
	10	0.1786	100	1.786	$m = \frac{15\ \Sigma\times 37.83 - 150 \times 2.559}{15 \times 2250 - 22500}$
	15	0.2564	225	3.846	
	20	0.3333	400	6.666	$m = 0.016321$
Series – 2	0	0.0004	0	0	$b = \frac{2250 \times 2.599 - 37.833 \times 150}{15 \times 2250 - 22500}$
	5	0.0956	25	0.478	
	10	0.1753	100	1.753	$b = 0.0074$
	15	0.255	225	3.825	
	20	0.3292	400	6.584	
Series – 3	0	-0.0003	0	0	
	5	0.095	25	0.475	
	10	0.1704	100	1.704	
	15	0.2531	225	3.7965	
	20	0.3221	400	6.442	

The standard calibration curve is shown in **Figure 2.** This linear regression line is constructed using the data shown in **Table 4**. The concentration (x value) of unknown sample is calculated using this calibration plot, based on the linear Equation (3). **Table 5** shows the measured absorbance (y value) of unknown analyte and the calculation of concentration of Pb (x value) in the digested solution and the soil sample.

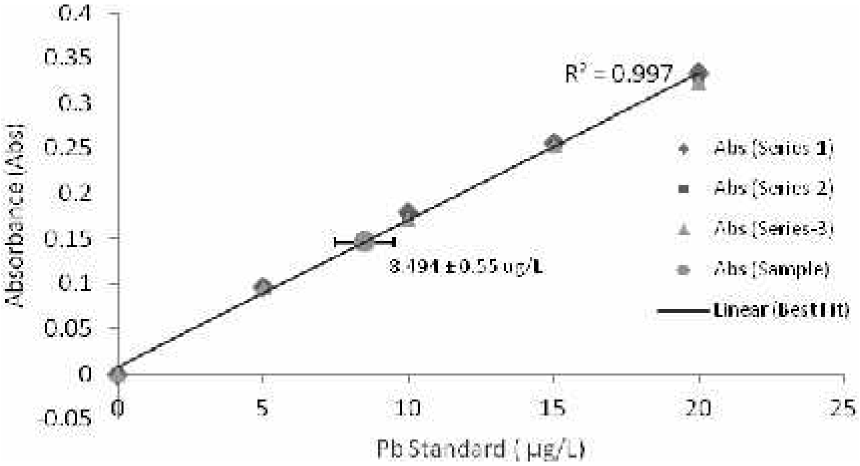

Fig. 2. Calibration plot of Standard Lead concentration *versus* Absorbance using GF-AAS; sample concentration (x =8.494 µg/mL) is measured with uncertainty (U_x = 0.55µg/mL) using LSM, is shown by circle dot with error bar.

Table 5: Calculation of unknown analyte concentration in digested solution and soil sample

Abs (replicate)	Average (Abs)	Pb (µg/L) in digested solution		Pb (µg/g) in soil sample
y_j	y	x = (y – b)/m		(c)
0.1422		8.26		$\mu\text{gPb/g soil (c)} = \frac{\text{Pb(g)}}{\text{sample taken (g)}} \times \frac{1}{10}$
0.1512		8.81		
	0.1460		8.494 ± 0.285	$= \frac{8.494}{0.5008} \times \frac{1}{10}$
0.1447		8.41		
				= 1.696

Table 6: Calculation of QC standard (CRM) concentration, repeatability & recovery

Abs (replicate)	**Average (Abs)**	**Calculated value of QC Std. of Pb (µg/L)**		**Interpretation**
0.1733		10.16		
0.1745	0.1732	10.24	10.16 ± 0.083	Standard Recovery = 101.6%
0.1718		10.07		

2. Uncertainty in unknown analyte (x) from Calibration Plot

Since the calculation of an x_i th-value from a given y_i th-value involves the use of both the slope (m) and intercept (b), all these values are subject to error. So, the error is expressed as Uncertainty in x (s_x) which is calculated from the Least Squares Method following the equations below [4,7]:

$$\text{Uncertainty in } x(s_x) = \frac{s_y}{|m|}\sqrt{\frac{1}{k}+\frac{1}{n}+\frac{(y-Y)^2}{m^2\sum(x_i-X)^2}} \tag{6}$$

where,

s_y = standard deviation of y_i th values (Eq. 5) from the calibration curve

$$= \sqrt{\frac{\sum(d)_i^2}{n-2}} \tag{7}$$

where, d_i = the vertical deviation for the point (x_i, y_i) in the calibration line

$$= y_i - \dot{y} = y_i - (mx_i + b)$$

where, $\dot{y}$ is the ordinate of the straight line when $\dot{x} = x_i$

$n - 2$ = degrees of freedom (δ)

and in Equation (6):

$\lvert m\rvert$	= absolute value of the slope
k	= number of replicate absorbance (y) measurement of the unknown
n	= the number of data points on the calibration line

y = mean value of absorbance of unknown sample

$\overline{Y}$ =mean value of for the points on the calibration line

x_i = individual values of x for the points on the calibration line

$\overline{X}$ = mean value of x for the points on the calibration line

Accordingly, using Equations (6 & 7), standard deviation in y_i th values and uncertainty in concentration of Pb in soil digest, x, was calculated and the results are computed in **Table 7 & Table 8:**

Table 7: Computation of standard deviation of y_i th values (s_y value from equation 7)

	Pb (µg/L)	Abs	d_i	d_i^2	$(x_i - \overline{X})^2$	Calculation of s_y
	x_i	y_i				
Series – 1	0	-0.0004	-0.0078	6.084E-05	100	$d_i = y_i - (mx_i + b)$, where values of m and b were obtained from Table 4. $\sum(x_i - \overline{X})^2 = 750$ $\sum d_i^2 = 0.00056$ $sy = \sqrt{\frac{\sum((d)_i^2)}{n-2}}$ $sy = \sqrt{\frac{0.00056}{15-2}}$ = 0.006573
	5	0.0955	0.0064	4.2163E-05	25	
	10	0.1786	0.0079	6.3787E-05	0	
	15	0.2564	0.0041	1.7472E-05	25	
	20	0.3333	-0.0005	2.7738E-07	100	
Series – 2	0	0.0004	-0.007	4.9E-05	100	
	5	0.0956	0.0065	4.3472E-05	25	
	10	0.1753	0.0046	2.1965E-05	0	
	15	0.255	0.0027	7.7284E-06	25	
	20	0.3292	-0.0046	2.1406E-05	100	
Series – 3	0	-0.0003	-0.0077	5.929E-05	100	
	5	0.095	0.0059	3.592E-05	25	
	10	0.1704	-0.0002	4.5511E-08	0	
	15	0.2531	0.0008	7.744E-07	25	
	20	0.3221	-0.0117	0.00013751	100	

Table 8: Computation of uncertainty (S_x) in x value (Pb in digested sample) from equation 6

Parameters	Calculated Value	Uncertainty in x (U_x) in unknown analyze)
n (number of data set, Table 7)	15	$Sx = \frac{s_y}{\lvert m \rvert}\sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y-Y)^2}{m^2\sum(x_i - \overline{X})}}$ $Sx = \frac{0.006573}{\lvert 0.0163}\sqrt{\frac{1}{3} + \frac{1}{15} + \frac{6.69E-06}{0.1998}}$ $S_x = 0.2547$ $U_x = \pm tS_x = (2.160)(0.2547)$ $U_x = \pm 0.552$
k (number of data of y_jth value, Table 5)	3	
$\overline{Y}$ (Table-4) (mean value of y_ith, Table 7)	0.171	
y (mean value of y_jth, Table 5)	0.1460	
$(y - \overline{Y})^2$	0.000604	
$\overline{X}$ (mean value of x_i, (150 ÷ 15), Table 7)	10	
$\sum(x_i - \overline{X})^2$ Σ (Table 7)	750	
$m^2\sum(x_i - \overline{X})^2$ (0.0163^2 X 750)	0.1998	
s_y (from Table 7)	0.006573	
± t(from t-Table, Statistical Reference[4,7])	2.16 at 95% CI	

3. Standard uncertainty budget in total analytical process for Pb in soil using GF-AAS Technique

The Uncertainty in the concentration **(U_c)** of Lead (Pb) in soil sample is the combination of the standard uncertainties from the following sources, summarized in Table 9:

Table 9: Standard uncertainty from different steps of analysis

Sources of standard uncertainty		Value (*f*)	Standard Uncertainty u(*f*)	Justification
x	Standard calibration plot	8.494 μg/L	0.552 μg/L	$U_x = \pm tS_x = 0.552$ from Table-8
p	Repeatability of sample conc. Due to matrix effect	8.494 μg/L	3.35/√3 = 1.93% = 0.0193 as RSD	RSD = 3.35% obtained from repeated measurement (n=3)
r	Recovery of standard concentration	10 μg/L	0.083/√3 = 0.048	SD = 0.083 (n=3), from replicate experiments
v	Volume of flask used for adjustment of final digested solution	100 mL	0.05/√3 = 0.0288 mL	$U_v = \frac{0.05ml}{\sqrt{3}}$ ± 0.05 mL*
m	Mass of soil sample taken for sample preparation	0.5008 g	0.00025 / 1.97 = 0.00013 g	$U_m = \frac{0.00025g}{1.97}$ **

* Standard uncertainty in volume, assuming rectangular distribution due to temperature variation

** Standard uncertainty mass in assuming 95% confidence level

Figure 3 shows the contribution of standard uncertainty from different steps of analytical process and it is pertinent that the contribution of uncertainty from calibration is pre-dominant.

4. Propagation of uncertainty and Expanded uncertainty

The propagation of uncertainties in the final concentration of lead in soil sample contributed from different sources is defined as Combined Uncertainty (U_c), which is calculated by using the formula:

$$\frac{U_c}{(c)} = \sqrt{\left(\frac{Ux}{x}\right)^2 + \left(\frac{Up}{x}\right)^2 + \left(\frac{Ur}{s}\right)^2 + \left(\frac{Um}{m}\right)^2 + \left(\frac{Uv}{v}\right)^2} \tag{8}$$

$$\frac{U_c}{(1.696)} = \sqrt{\left(\frac{0.552}{8.494}\right)^2 + \left(\frac{0.0193}{8.494}\right)^2 + \left(\frac{0.048}{10}\right)^2 + \left(\frac{0.00013}{0.508}\right)^2 + \left(\frac{0.0288}{100}\right)^2} \quad \text{(from Table-9)}$$

$$= \sqrt{0.0043}$$

$$Uc = 1.696\sqrt{0.00043} = 0.1106$$

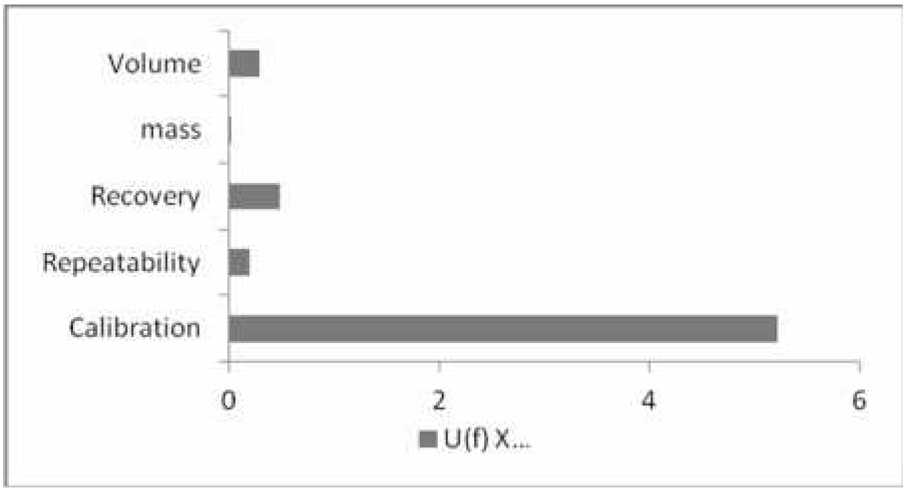

Fig. 3. Contribution of standard uncertainty from different sources (Table-9); Uncertainty from calibration is predominant

Expanded Uncertainty (U_E)

The Expanded Uncertainty is expressed as multiplication of combined uncertainty with a coverage factor based on desired confidence interval (CI). Generally, the coverage factor (K) is considered 2 for 95% confidence level. So, Expanded uncertainty (U_E) for Pb in soil is 2 x U_c = 2 x 0.1046 = 0.2092µg /g soil

5. Lead (Pb) concentration in soil with expanded uncertainty

The final result of Pb analysis in Soil is expressed as: **X ± U_E (unit) at 95% confidence level.** In the given example, the final result is 1.696 ± 0.21 µg Pb /g soil (at 95% CL). So, the final value, if expressed as mg/kg, would be**: 1.69 ± 0.21 mg Pb/kg soil (at 95% confidence limit).**

Process steps of measurement uncertainty for Pesticide in wastewater

6. Construction of linear calibration plot using LSM (value of m & v) and measured unknown concentration

Table 10 shows the Excel spreadsheet for computing linear calibration for Parathion Methyl pesticide as an example based on the data shown in Table 2. **Table 10** shows the Excel command formula *f*(*x*) next to the cell of each calculated value for each variable of LSM method. The symbols of all parameters of Least Squares Equation are the same as was discussed in the first exercise.

Table 10: Excel Spreadsheet for calculation of the variable of LSM (m & b value) & measurement uncertainty (U_x)

	A	B	C	D	E	F	G	H	I	J
1		Parathion Methyl					Sample (wastewater)			
2		Std µg/ mL	Mean P. A.*					Mean P. A.*		
3		x_i	y_i x 10^{-4}	$(x_i - \bar{X})^2$	d_i^2			y_j x 10^{-4}		
4		2.5	0.9915	169	0.360414			9.7185		
5		5	2.5274	110.25	0.042849			10.5872		
6		10	4.6199	30.25	2.430132			12.1314		
7		20	14.7461	20.25	0.723238			13.5771		
8		40	29.2304	600.25	0.00978			13.517		
9	n	5	=COUNT(B4:B8)							
10	$\bar{X}$	15.5	=AVERAGE(B4:B8)					*P.A = Peak Area		
11	$\sum(x_i - \bar{X})^2$	930	=SUM(D4:D8), where D4 = (B4-B10)^2 … … … …							
12			… … D8 = (B8-B10)^2							
13	$\bar{y}$	10.4231	=AVERAGE(C4:C8)							
14	m	0.772	=SLOPE(C4:C8,B4:B8)							
15	b	-1.538	=INTERCEPT(C4:C8,B4:B8)							
16	$\sum(d_i)^2$	3.57	=SUM(E4:E8), where, E4 = (C4-($B$14*B4+$B$15))^2 … …							
17			… … … E8 = (C4-($B$14*B8+$B$15))^2							
18	δ	3	=B9-2							
19	S_y	1.09	=SQRT((B16/B18))							
20										
21	y	11.91	=AVERAGE(H4:H8)							
22	$x^{\#}$	17.42	=(B21-B15)/B14		$^{\#}$ Concentration of Parathion Methyl					
23	k	5	=COUNT(H4:H8)							
24	$S_y/\lvert m \rvert$	1.413	=B19/ABS(B14)							
25	$(y - \bar{Y})^2$	2.200	=(B21-B13)^2							
26	$m^2 \sum(x_i - \bar{X})^2$	553.81	=(B14^2)*B11							
27	S_x	0.921	=J12*SQRT((1/J11)+(1/B9)+(J13/J14)^2/(B14)^2*B11)							
28	*t*	3.182	=TINV(0.05,B18), considering 95% CI							
29	U_x	2.930	=J15*J16							
30										

The concentration of Parathion Methyl in the PT sample is shown in Table 11 based on the data of Table 3 and that of Excel spreadsheet of Table 10. The concentration of Malathion is also shown in Table 11 based on the data of Table 3 and that of spreadsheet calculation following **Table 10**.

Table: 11: Parameters of calibration plot and the concentration of two pesticides (model calculation)

PT Sample	Slope (m)	Intercept (b)	Concentration (x) in 1 ml extract (μg/mL)	Conc (x) in diluted (1000 mL) wastewater (μg/L)	Uncertainty (U_x)
Parathion Methyl	0.7717	-1.538	17.422	17.422 (RSD=9.93%, n=5)	± 2.930
Malathion	0.9483	-1.227	15.397	15.397 (RSD=9.72%, n=5)	± 3.799

7. Uncertainty in unknown analyte (x) and QC standard from Calibration Plot

In **Table 10,** the uncertainty of concentration (U_x) of Parathion Methyl, computed equation 6, is ± 2.930 and for Malathion, it is ± 3.799. In **Table 12**, repeatability in concentration and the recovery of the CRM standard (10 ppm) is computed. It is noted that, the percent recovery of the standard is about 25% lower than the certified value. The uncertainties for CRM of both pesticides are calculated from the repeatability of the standard.

Table 12: Recovery and repeatability of CRM – QC standard (10 ppm each)

	Recovery from standard	Standard deviation	Number of data points	Uncertainty
Parathion Methyl (10 ppm)	7.64 (76.4%)	0.25	8	$0.25/\sqrt{8} = \pm 0.088$
Malathion (10 ppm)	7.46 (74.6%)	0.22	7	$0.22/\sqrt{7} = \pm 0.083$

8. Standard uncertainty budget in total analytical process for Pesticide analysis in wastewater

In **Table 13,** the contributions of standard uncertainties from different sources are computed for both pesticides.

Table 13: Standard uncertainty from different analytical steps

		Parathion Methyl		Malathion	
	Sources of standard uncertainty	Value (f)	Standard Uncertainty u(f)	Value (f)	Standard Uncertainty u(f)
x	Standard calibration plot	17.422 ppm	2.930 ppm	15.397 ppm	3.799 ppm
p	Repeatability of sample conc. due to matrix effect	17.422 ppm	$9.93\%/\sqrt{5}=4.44\%$ = 0.0441	15.397	$9.72\%/\sqrt{5} = 4.35\ \%$ = 0.0435
r	Recovery of standard concentration (CRM)	10 ppm	0.088 ppm	10 ppm	0.083 ppm
$V1$	Volume of Pipette for dispensing sample from the ampoule*	1 mL	$0.01/\sqrt{3} = 0.0058$	1 mL	$0.01/\sqrt{3} = 0.0058$
$V2$	Volume of flask used for preparing diluted sample*	1000 mL	$0.5/\sqrt{3} = 0.289$	1000 mL	$0.5/\sqrt{3} = 0.289$
$V3$	Volume of Pipette for preparing extractant*	1 mL	$0.01/\sqrt{3} = 0.0058$	1 mL	$0.01/\sqrt{3} = 0.0058$

* The standard uncertainty in volume is assuming rectangular distribution for the temperature variation

9. Propagation of uncertainty & Expanded Uncertainty

The propagation of uncertainty from different sources identified in Table 13 are used to calculate combined (U_c) and Expanded uncertainty (U_E) for both the compounds of OPP, which is summarized in **Table 14**.

Table 14: Calculation of Combined Uncertainty (U_c) and Expanded Uncertainty (U_E)

	Parathion Methyl			Malathion		
	f	u(*f*)	(u(*f*)/*f*)^2	*f*	u(*f*)	(u(*f*)/*f*)^2
Calibration	17.422µg/mL	2.93	0.0283	15.397µg/mL	3.799	0.0609
Repeatability	17.422µg/mL	0.0441	0.0000	15.397µg/mL	0.044	0.0000
Recovery	10µg/mL	0.88	0.0077	10µg/mL	0.88	0.0077
Volume (1 mL)	1 mL	0.0058	0.0000	1 mL	0.006	0.0000
Volume (1000 mL)	1000 mL	0.289	0.0000	1000 mL	0.289	0.0000
Volume (1 mL)	1 mL	0.0058	0.0000	1 mL	0.006	0.0000
$\Sigma(U_f/f)^2$			0.0361			0.0687
Propagation of Uncertainty (U_c / c) = $\sqrt{\Sigma(U_f/f)^2}$			0.1900			0.2621
Concentration of diluted wastewater (C)			17.42µg/L			15.4µg/L
Combined Uncertainty U_c			3.31 µg/L			4.04 µg/L
Expanded Uncertainty, U_E, (K=2 at 95% CI)			6.62 µg/L			8.08 µg/L

From Table 14, it is apparent that the major contribution to uncertainty comes from the standard calibration and recovery of the analyte in replicate analysis inherent to GC-MS instrumentation. Finally, the concentration of Parathion Methyl in diluted PT sample was found to be **17.4 ± 6.6 «g/L** and that of Malathion was **15.4 ± 8.1 «g/L**.

10. Comparison of Lab and PT Reports

The calculated test results of two OPP compounds and the measurement uncertainty are compared with the PT reports in Figure 4. It is observed that the results submitted by this laboratory are slightly positively biased from the assigned value of the PT provider. The relative uncertainties of Parathion Methyl and Malathion from this laboratory are **38%** and **52.5 %** respectively. This is relatively lower than the PT interlaboratory variation of 60% for each component.

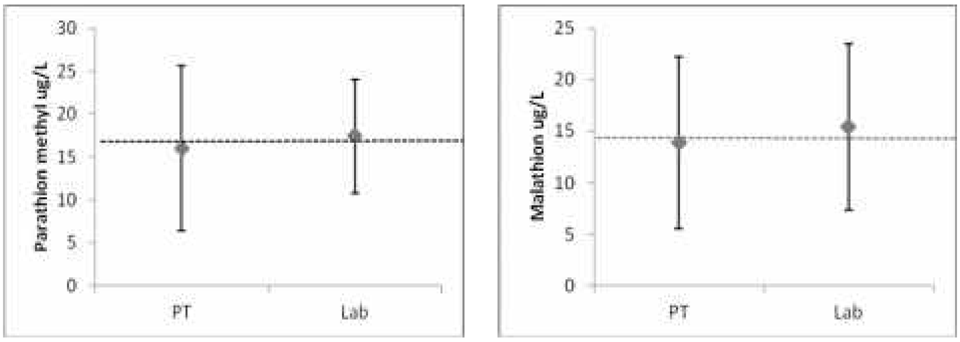

Fig. 4. Comparison of Proficiency Testing (PT) report and Lab report with measurement uncertainty for two OPP compounds

CONCLUSION

Analytical measurement is a constant need of a modern society as a public health advisory for assuring required quality specifications of consumers' products such as food, drinks and pharmaceuticals. In certifying materials for specific use/consumption, analytical testing laboratories all over the world report test results with assigning a level of uncertainty without which the results are not considered as scientific facts. In the context of Bangladesh, however, most of the testing laboratories report analytical results without quoting uncertainties associated with the results. Such test results either in scientific research or in international trade and commerce are not of any significance and as such are not any more acceptable.

To overcome this limitation in our efforts in materials testing for quality specifications, the modern concepts of quantifying uncertainties in measurements are introduced and how these concepts are used to quantify measurement uncertainties in chemical and biochemical analyses is illustrated with field sample analysis using instrumental methods such as atomic absorption spectroscopy and GC-MS spectrometry. This communication would thus enable testing laboratories operating in Bangladesh to report their analytical test results with a reliable statement on the level of uncertainties associated with the test results.

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