Review of Methodology for Estimation of Labile Organic Carbon in Reservoirs and Lakes for GHG Emission

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Received 11 Sept 2013, Revised 23 Jan 2014, Accepted 23 Jan 2014
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Abstract
Organic carbon (OC) can be classified into three main categories: labile, semi labile and refractory. The area of research is primarily focused on the labile organic carbon (LOC) as it is considered highly reactive indicator of green house gas (GHG) emission from reservoirs and lakes. The CO₂ or CH₄ release from sediment OC depends on its source and temperature of the reservoirs. The several analytical methods are available to determine and evaluate LOC based on physical, chemical and biochemical methods. A review of the advantages and disadvantages of each technique has indicated that none of the methods can be used to determine LOC precisely either because a part of the LOC is not involved or its further characterization is apparently missing. The approach is an omnipotent tool for the measurement of LOC. The present paper reviews the methodologies for estimating LOC and other types of organic carbon like LOC which plays crucial role in estimating the GHG emissions from reservoirs /lakes and lays the emphasis only on LOC (DOC, DIC and POC) due to its rapid degradation for the release of GHG compared to other ‘C’ types.

Keywords: Emission, green house gas, labile organic carbon.

1. Introduction
Lake and reservoirs and their sediments, generally rich in organic matter (OM) consist of labile and refractory compounds which undergo complex processes like degradation, heterotrophic utilization, transformation, accumulation and export [1]. Refractory organic compounds (ROC) like humic and fulvic acids, structural carbohydrates and “black” carbon account for most of the sedimentary OM [2,3] while the labile fraction (LF) of OM mainly consists of simple and/or combined organic molecules like carbohydrates, lipids and proteins which may undergo mineralization [4,5] and result in green house gas (GHG) production in water-bodies. Few labile compounds may be resistant to degradation due to complex interactions occurring within the sedimentary matrix and/or ROC [6]. Though, the measurement of the labile fraction of sedimentary organic matter is a difficult task and as yet, no widely accepted method is available [7]. Few authors have estimated the LF of sedimentary OM by determining the main biochemical organic compounds like carbohydrates, proteins and lipids which may be easier to digest and assimilate [8,11]. The approach is impractical as the total carbohydrate assessment can be used to discriminate between highly refractory and easily degradable compounds [12]. Further, the laboratory approach based on an enzymatic hydrolysis of sediment samples is also proposed to mimic the OM degradation in deposit [13, 15].

The present paper discusses only the labile organic carbon (LOC) which undergoes rapid degradation resulting in rapid GHG emissions from reservoirs and lakes. The paper also deals with a new approach to organic matter classification and reviews the principle methods for separation, quantification and evaluation of LOC due to rapid GHG emissions from reservoirs and lakes compare to other types of carbons.

2. Classification of carbons
Carbons may be classified into 3 types on the basis of its function as:

a) Labile organic carbon (LOC) – consisting of low molecular weight (LMW) compounds that support heterotrophic bacterial growth.

b) Semi-labile organic carbon (SLOC) – consists of high molecular weight (HMW) and LMW compounds resistant to rapid microbial degradation (e.g. carbohydrates, partially hetero-polysaccharides).

c) Refractory organic carbon (ROC) – dominated by the presence of LMW compounds resistant to microbial re-mineralization. It is photo-chemically active material that is transformed to biologically labile material.
Table 1 gives the salient features of organic carbons in reservoirs/lakes based on literature.

### Table 1: Salient features of organic carbon in reservoirs/lakes

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Types of carbon</th>
<th>Forms of carbon</th>
<th>Fraction of total carbon (%)</th>
<th>Features</th>
<th>Turn-over time (years)</th>
<th>Degradation rate</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Labile carbon</td>
<td>Soluble fresh residues</td>
<td>0.5-5</td>
<td>Root exudates and microbes</td>
<td>&lt;0.1</td>
<td>Very high</td>
<td>[16]</td>
</tr>
<tr>
<td>2</td>
<td>Flora and fauna</td>
<td>1-10</td>
<td>----</td>
<td>&lt;5</td>
<td>High</td>
<td>[16,17]</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>POC (particulate organic carbon)</td>
<td>1-40</td>
<td>&gt;53 µm, particle size</td>
<td>&lt;10</td>
<td>High</td>
<td>[18-20]</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Light fraction</td>
<td>1-30</td>
<td>&lt;1.6-2 g/cm³ density</td>
<td>&lt;10</td>
<td>Medium</td>
<td>[17]</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Semilabile carbon</td>
<td>Humus</td>
<td>30-50</td>
<td>Total organic carbon (TOC), POC</td>
<td>10-200</td>
<td>Medium</td>
<td>[18]</td>
</tr>
<tr>
<td>6</td>
<td>Clay-complexes carbon</td>
<td>30-60</td>
<td>&lt;2 µm, size particle</td>
<td>10-100</td>
<td>Medium</td>
<td>[17]</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Refractory carbon</td>
<td>Charcoal</td>
<td>1-30</td>
<td>Resistant to chemical oxidation</td>
<td>&gt;100</td>
<td>Slow</td>
<td>[18]</td>
</tr>
<tr>
<td>8</td>
<td>Phytoliths</td>
<td>1-30</td>
<td>Oxidizable at 1300°C</td>
<td>Millenia</td>
<td>Very slow</td>
<td>[21, 22]</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Carbonates</td>
<td>0-30</td>
<td>Release of CO₂ by acidification</td>
<td>&gt;1000</td>
<td>Very slow</td>
<td>[23-25]</td>
<td></td>
</tr>
</tbody>
</table>

The table shows that the rate of degradation of LOC is rapid as compared to other types of organic carbon e.g. SLOC and ROC with very low turn over time (<10 years). LOC is very small (0.5-5%) compared to SLOC & ROC with relatively higher rate of degradation with short period of time <0.1 to <10 years yielding maximum GHG from reservoirs and lakes. Table 2 provides the details of different types of carbons, their salient features & significance from the point of GHG emissions.

### 3. Transport of total carbon from catchment into reservoirs/lakes

Allochthonous or autochthonous carbon follows three major pathways to reach in lakes and reservoirs and the relative importance of each pathway determines the source or sink of GHGs in aquatic environment. These pathways are: (1) Dissolved organic carbon (DOC) and POC are transported from water column to the sediment via flocculation into biological material and sedimentation of particulate organic matter. (2) DOC and POC are degraded by photochemical and microbial processes resulting in the mineralization of organic carbon (OC) to CH₄ and CO₂ & (3) carbon compounds flow passively downstream to river, groundwater and marine systems.

### Table 2: Types of carbon and its contribution to GHG emissions from reservoirs/lakes

<table>
<thead>
<tr>
<th>Sample</th>
<th>Symbol</th>
<th>Description</th>
<th>Significance with respect to GHG Emissions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic carbon</td>
<td>OC</td>
<td>The soils are predominantly rich in calcite and dolomite [26].</td>
<td>----</td>
</tr>
<tr>
<td>Inorganic carbon</td>
<td>IC</td>
<td>All carbon found in any compound and particle TC=TIC+TOC</td>
<td>No contribution to GHG emissions</td>
</tr>
<tr>
<td>Liquid matrix</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total carbon</td>
<td>TC</td>
<td>All carbon in the carbonate, bicarbonate, dissolved CO₂ in water bodies [27]. Quantity depending on pH, temperature and partial pressure of CO₂ [28]. TIC=DIC+PIC</td>
<td>No contribution to GHG emissions</td>
</tr>
<tr>
<td>Total inorganic carbon</td>
<td>TIC</td>
<td>All organic species that are soluble [29] or pass through a filter of 0.45 µm [27]. DOC = VOC + NPDOC</td>
<td>Contribute to CH₄ &amp; CO₂ production</td>
</tr>
<tr>
<td>Dissolved inorganic carbon</td>
<td>DIC</td>
<td>Suspended particle material</td>
<td>No contribution to GHG emissions</td>
</tr>
<tr>
<td>Particulate inorganic carbon</td>
<td>PIC</td>
<td>Indication of water contamination by synthetic organic compounds [28, 29]; chemical characterization [30]; estimation of carbon content of soil [31]; carbon fluxes in aquatic systems [32]. TOC= DOC+POC or TOC= NPOC + VOC</td>
<td>Contribute to CH₄ &amp; CO₂ production</td>
</tr>
<tr>
<td>Total organic carbon</td>
<td>TOC</td>
<td>All organic species that are soluble [29] or pass through a filter of 0.45 µm [27]. DOC = VOC + NPDOC</td>
<td>Contribute to CH₄ &amp; CO₂ production</td>
</tr>
<tr>
<td>Dissolved organic carbon</td>
<td>DOC</td>
<td>The soils are predominantly rich in calcite and dolomite [26].</td>
<td>Contribute to CH₄ &amp; CO₂ production</td>
</tr>
</tbody>
</table>

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carbon & 0.2–10-µm filter [33] & production \\
Volatile organic carbon & VOC & Low boiling (<100 °C) [34], and LMW compounds. & No contribution to GHG emissions \\
Purgeable organic carbon & POC & OC released by sparging. & Contribute to CH₄ & CO₂ production \\
Non-purgeable organic carbon & NPOC & Not removed by sparging [29]. NPOC = NPDOC + POC & No contribution to GHG emissions \\
Non-volatile organic carbon & NVOC & & No contribution to GHG emissions \\
Non-purgeable dissolved organic carbon & NVDOC & & No contribution to GHG emissions \\
Solid matrix Solid & Total carbon & TC & All carbon in solid form, TC = TIC + TOC & ---- \\
Total inorganic carbon & TIC & & ---- \\
Total organic carbon & TOC & & ---- \\
Volatile organic carbon & VOC & & ---- \\
Non-volatile organic carbon & NVOC & & ---- \\
Acid soluble organic carbon & ASOC & It is lost during separation of the spent acid [35] up to 45%. Increases almost with the % of CaCO₃ in the sample [36]. & ---- \\
Acid insoluble organic carbon & AIOC & TOC = AIOC + ASOC [37] & ---- \\
Oxidizable carbon & OXC & Easily oxidizable OC, not stabilized in organic-mineral complexes [38] & ---- \\
Soil organic matter & SOM & Organic materials that go along with soil particles through a 2 mm sieve [26, 39]. Contribute to CH₄ & CO₂ production & ---- \\

It is clear from the Table that DIC, DOC and POC are mainly responsible for GHG emission in the reservoirs and lakes while other carbon types like TIC, PIC, VOC, NVOC etc. do not contribute to GHG emission. The former are more important than the later from GHG emissions point of view. The DIC, DOC & POC can, therefore, form valuable basis to assess GHG emission potential of reservoirs/lakes.

POC and DOC are converted into CH₄ and CO₂ through the organic matter degradation in sediments and water column. If CH₄ concentration above methane solubility (21-35 mg/l at normal temp and pressure), it is emitted directly to the atmosphere by bubbling in shallow regions of the reservoirs (Figure 1). Mineralization of DOC and release of DIC are linked to lake stratification while the increase in temperature leads to anoxic conditions which reduce the rate of DOC mineralization.

4. Significance of labile organic carbon (LOC)
The labile fraction of OC consists of micro-organisms, plant and soil fauna at different stages of degradation resulting in easily decomposable non-humic organic substances like carbohydrates, proteins, organic acids, amino acids, waxes, and other non-specific compounds [40]. LMW soil organic matter is gaining the attention of researchers all over the world due to the presence of monosaccharides, amino acids and organic acids [41–45]. The rate of decomposition or mineralization is governed by the presence of temperature, porosity, pH, nature of the compounds present and their availability for micro-organisms [46].
5. Methods to determine LOC

Two types of analytical method are used frequently for the analysis of LOC: i) physical, chemical and biochemical analysis of the non-living substrate and ii) determination of the microbial activity. Table 3 reviews the methods for separation and/or evaluation of the LF. Each method is briefly detailed below:

5.1. Physical methods

These consist of POC, Densitometric separation, Dissolved organic matter (DOM) and Water-extractable organic matter (WEOM). Each method is further briefly discussed below:

5.1.1. Particulate organic carbon (POC)

It consists of plants or faunal residues, sometime referred as inert charcoal.

5.1.2. Densitometric separation

Density fractionation is based on different densities of mineral fraction (> 2) and organic matter (< 1.6 g/cm³).

5.1.3. Water-extractable organic matter (WEOM) and dissolved organic matter (DOM)

DOM remains naturally dissolved in soil-water solution like saccharides, amino acids, and aminosugars. WEOM consists of organic matter extracted from soil under laboratory conditions. It has wide spectrum of extracted compounds like hemicelluloses [47] but neither DOM nor WEOM is the substrate for micro-organisms.

5.2. Chemical methods

These are oxidation and acid hydrolysis as discussed below:

5.2.1. Oxidation method

Wet oxidation is a popular method for the determination of organic matter in soil. Chan et al. [48] modified the classical Walkley-Black [49] oxidation method while Strosser [50] has proposed another “sequential oxidation method”. None of the method completely oxidizes OC. In Walkley-Black method, 90% of OC while in sequential oxidation method, only 75% of OC is oxidized.

5.2.2. Acid hydrolysis

Acid Hydrolysis retards the degradation of organic matter by extracellular enzymes of soil micro-organisms. Rovira and Vallejo [51] reported that three-step H₂SO₄ hydrolysis is more extensive. H₂SO₄ is found more effective than HCl for the hydrolysis of organic matter e.g. plant tissues [52, 53].

5.3. Biochemical methods

It consists of microbial biomass carbon (MBC), soil respiration and biochemical oxygen demand (BOD) methods as discussed below.

5.3.1. Microbial biomass carbon (MBC)

Soil MBC is used to evaluate of LF [54, 55] but it does not consider species composition of microbial communities or their enzymatic capacity [56].

5.3.2. Biochemical oxygen demand (BOD) and soil respiration

The LF can be measured by CO₂ released by micro-organisms using respiration test. Kolar et al. [57] have proposed a method on the basis of BOD. However, none of methods gives a complete assessment of LF without additives.

<table>
<thead>
<tr>
<th>Name of method (A)</th>
<th>Types of carbon</th>
<th>Principle</th>
<th>Fractions</th>
<th>Advantages (+) disadvantages (-)</th>
<th>Ref.</th>
</tr>
</thead>
</table>
| Physical          | POC             | Particles unable to pass through wet sieving | TOC in size fraction >53 µm | • Easy performance (+)  
• Inadequate knowledge about properties and function (-)  
• Possibility to involve inert charcoal (-)  
• Only quality characterization (-) | [58, 59] |
| Size fractionation | Light fraction | Separation in heavy liquid solution e.g. sodium polytungstate | Light : <1.6 g/ml  
Medium : 1.6-2.0 g/ml  
Heavy : > 2.0 g/ml | • Easy performance (+)  
• Only quality characterization (-) | [60] |

Table 3: Physical, chemical and biochemical method for labile organic carbon analysis
<table>
<thead>
<tr>
<th>Dissolved organic matter (DOM)</th>
<th>DOC</th>
<th>TOC dissolved in soil solution</th>
<th>TOC in field moist soil sample after centrifugation by 16,000 g for 30 min. at 4 °C (large stone removed &amp; aggregates broken by hand).</th>
<th>Evaluation of the actual level of organic matter scattered in water solution (+)</th>
<th>Only quality characterization (-)</th>
<th>[62]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extractable organic matter (WEOM)</td>
<td>Hot water soluble carbon</td>
<td>Hot water-extractable carbon</td>
<td>TOC in extract: 60 min. gentle boiling in distilled water (DW).</td>
<td>Easy performance with good results (+)</td>
<td>Only quality characterization (-)</td>
<td>Does not involve complete labile fraction (-)</td>
</tr>
<tr>
<td>Cold water soluble carbon</td>
<td>Cold water-extractable carbon</td>
<td>TOC in extract: 30 min at 20 °C shaking in DW</td>
<td>Easy performance (+)</td>
<td>Bad result (-)</td>
<td>Only quality characterization (-)</td>
<td>[64]</td>
</tr>
<tr>
<td>Water soluble carbon</td>
<td>Water soluble carbon</td>
<td>TOC in extract: 1 h at 20 °C shaking in DW, followed by centrifugation and filtration.</td>
<td></td>
<td></td>
<td></td>
<td>[66]</td>
</tr>
</tbody>
</table>

(B) Chemical

<p>| Oxidation | • Carbon oxidized with potassium | Use KMnO₄ as oxidizing agent | Fraction I = C oxidized by 333 mM KMnO₄ | Fraction II = TOC – fraction I | Easy performance (+) | Only quality characterization (-) | [67-70] |
|-----------|--------------------------------|-----------------------------|------------------------------------------|-------------------------------|---------------------------------------------------------------------------------|------|
| • Modified Walkley-Black method | C oxidized by 0.167 M K₂Cr₂O₇ | | Fraction 1 : 6 M H₂SO₄ - F1 | Fraction 2 : 9 M H₂SO₄ - F2 | | | [69, 71-73] |
| | | | Fraction 3 : 12 M H₂SO₄- F2 | Fraction 4 : TOC - F3 | | | |
| Sequential oxidation | C oxidised with K₂Cr₂O₇ + H₂SO₄ mixture (45 min. at 125 °C) and retitration with 0.1 M Fe²⁺ | | Fraction 1 =0.017 M K₂Cr₂O₇ + 2.25 M H₂SO₄ | Fraction 2 =0.033 M K₂Cr₂O₇ + 4.50 M H₂SO₄ | | | [50] |
| | | | Fraction 3 =0.0500 M K₂Cr₂O₇ + 6.75 M H₂SO₄ | Fraction 4 =0.067 M K₂Cr₂O₇ + 9.00 M H₂SO₄ | | | |
| Dissolved organic matter (DOM) | DOC | K₂SO₄ | Extraction of soil (0-6 h) with either distilled water or 0.5 M K₂SO₄ (20 °C at 4 °C or in the presence of an inhibitor of microbial activity (HgCl₂ and Na-azide)). | | Only quality characterization (-) | [74] |
| Acid hydrolysis | Decomposable and resistant plant material (lignin and fat resin) | TOC in H₂SO₄ hydrolysate | Labile pool 1 : 2.5 M H₂SO₄ (30 min. at 105 °C ) | Labile pool 1 : 13 M H₂SO₄ (20 °C overnight next dilution to 1 M H₂SO₄, 3h at 105 °C ) LP I | Very sensitively distinguish fractions (+) | Suitable to various substrates (+) | [75, 76] |
| | | | Recalcitrant pool : TOC – LP II | | | | |</p>
<table>
<thead>
<tr>
<th>(C) Biochemical</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Micro-organism activity</strong></td>
<td><strong>Microbial biomass carbon</strong></td>
<td><strong>Microbial biomass carbon</strong></td>
</tr>
<tr>
<td>C difference between fumigated and non-fumigated soil sample</td>
<td>Fumigation with chloroform for 24 h, followed by extraction with 0.5 M K$_2$SO$_4$ for 2 h (shaking)</td>
<td>Good concept for determination of micro-organism amount (+)</td>
</tr>
<tr>
<td></td>
<td>C determined by chemical oxygen demand (COD) or TOC</td>
<td>No estimation of enzymes activity (-)</td>
</tr>
<tr>
<td></td>
<td>Basal soil respiration</td>
<td>True mineralisable organic matter (+)</td>
</tr>
<tr>
<td></td>
<td>CO$_2$ evolved from soil during incubation</td>
<td>Takes short time (+)</td>
</tr>
<tr>
<td></td>
<td>20 h incubation at 28°C</td>
<td>Charactarises both quality and quantity (+)</td>
</tr>
<tr>
<td></td>
<td>Evolved CO$_2$ estimated</td>
<td>Only most labile compound are mineralized (-)</td>
</tr>
<tr>
<td><strong>Micro-organism activity &amp; substrate quality</strong></td>
<td><strong>Mineralizable C</strong></td>
<td><strong>Mineralizable C</strong></td>
</tr>
<tr>
<td>CO$_2$ evolved from soil during incubation</td>
<td>24 day incubation at 25°C</td>
<td>True mineralization organic matter (+)</td>
</tr>
<tr>
<td></td>
<td>CO$_2$ evolved trapped in 1.0 M NaOH</td>
<td>Charactarises both quality and quantity (+)</td>
</tr>
<tr>
<td></td>
<td>Surplus of alkali titrated with 1.0 M HCl</td>
<td>Takes long time (-)</td>
</tr>
<tr>
<td><strong>Mineralizable N</strong></td>
<td>NH$_4^+$ evolved during anaerobic incubation</td>
<td>Charactarises both quality and quantity (+)</td>
</tr>
<tr>
<td></td>
<td>7 days incubation at 40°C</td>
<td>Only most labile compound are mineralized (-)</td>
</tr>
<tr>
<td></td>
<td>NH$_4^+$ is determined as total organic nitrogen (TON)</td>
<td></td>
</tr>
<tr>
<td><strong>BOD and K$_1$</strong></td>
<td>Biochemical oxygen demand and reaction rate constant</td>
<td>Easy performance (+)</td>
</tr>
<tr>
<td></td>
<td>% day BOD$_5$ determined manometrically</td>
<td>Charactarises both quality and quantity (+)</td>
</tr>
<tr>
<td></td>
<td>Calculation of reaction rate constant</td>
<td>Cost retentively high (-)</td>
</tr>
<tr>
<td><strong>(D) Instrumental</strong></td>
<td><strong>TOC</strong></td>
<td><strong>DOC, DIC and POC</strong></td>
</tr>
<tr>
<td>TOC</td>
<td>Removal of inorganic carbonates and degassing of CO$_2$</td>
<td>OC is oxidized in a furnace followed by direct determination of the evolved CO$_2$.</td>
</tr>
<tr>
<td></td>
<td>Decarbonation : 20 cm$^3$ of 0.5 M HCl to 10 g of sediment in a petri dish</td>
<td></td>
</tr>
<tr>
<td></td>
<td>leaving for 30 min</td>
<td>Coefficient of variation (CV): 3 % (+)</td>
</tr>
<tr>
<td></td>
<td>Oven at 105°C for 1 h.</td>
<td>Loss of OC due to decarbonation (-)</td>
</tr>
<tr>
<td></td>
<td>2 mg of dried at 105°C was weighed in a tin capsule using a Leco 650 microbalance</td>
<td>Formation of hygroscopic chloride (-)</td>
</tr>
<tr>
<td><strong>Carbon analyzer (Dry combustion)</strong></td>
<td><strong>DOC, DIC and POC</strong></td>
<td></td>
</tr>
<tr>
<td>DOC, DIC and POC</td>
<td>OC is oxidized in a furnace followed by direct determination of the evolved CO$_2$.</td>
<td>Accurate and quick result (+)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Expensive instrument (-)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Consumables, need pure O$_2$ gas (-)</td>
</tr>
</tbody>
</table>

Where:
Quality: decomposability or other related characteristics
Quantity: size and amount of organic matter in fraction

Despite the large number of methods for the estimation of LOC, only chemical method viz. acid hydrolysis, Modified Walkley-Black and Sequential Oxidation method may be considered as relatively better to obtain reliable results with high recovery of organic carbon and more reproducible results in terms of quality and quantity. The review of the methods has
revealed that no suitable method is available for the estimation of LOC and therefore there, is considerable scope for developing suitable method for it.

Conclusions
The review reveals that CO₂ or CH₄ release from sediment OC depends on the source of OC and temperature of the reservoir. LOC of the sediment undergoes degradation processes under anoxic condition and releases CO₂ and CH₄ in water column. On the basis of the above, it is concluded that LOC (DOC, DIC and POC) is mainly responsible for GHG emissions due to its high degradation rates in reservoirs/lakes compared to semi labile and refractory carbon. Out of the methods discussed above, no method is available that can be precisely used to estimate the LOC. This necessitates the development of a suitable method which should yield precise and reproducible results with less time, efforts and cost.

References

(2014) : www.jmaterenvironsci.com