



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

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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:

- Labile organic carbon (LOC) – consisting of low molecular weight (LMW) compounds that support heterotrophic bacterial growth.
- 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).
- Refractory organic carbon (ROC) – dominated by the presence of LMW compounds resistant to microbial remineralization. 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

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

The Table shows that 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

Sample	Symbol	Description	Significance with respect to GHG Emissions
Organic carbon	OC		-----
Inorganic carbon	IC	The soils are predominantly rich in calcite and dolomite [26].	-----
Liquid matrix			
Total carbon	TC	All carbon found in any compound and particle TC=TIC+TOC	No contribution to GHG emissions
Total inorganic carbon	TIC	All inorganic carbon is available in the carbonate, bicarbonate, dissolved CO ₂ in water bodies [27]. Quantity depending on pH, temperature and partial pressure of CO ₂ [28]. TIC=DIC+PIC	-----
Dissolved inorganic carbon	DIC		Contribute to CH ₄ & CO ₂ production
Particulate inorganic carbon	PIC	Suspended particle material	No contribution to GHG emissions
Total organic carbon	TOC	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	Contribute to CH ₄ & CO ₂ production
Dissolved organic carbon	DOC	All organic species that are soluble [29] or pass through a filter of 0.45 µm [27]. DOC = VOC + NPDOC	Contribute to CH ₄ & CO ₂ production
Particulate organic	POC	Suspended particles, moieties that are kept back by a	Contribute to CH ₄ & CO ₂

carbon		0.2–10- μ m 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 CH ₄ & CO ₂ 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	NPDOC		No contribution to GHG emissions
Solid matrix Solid	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 CaCO ₃ 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 CH ₄ & CO ₂ 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.

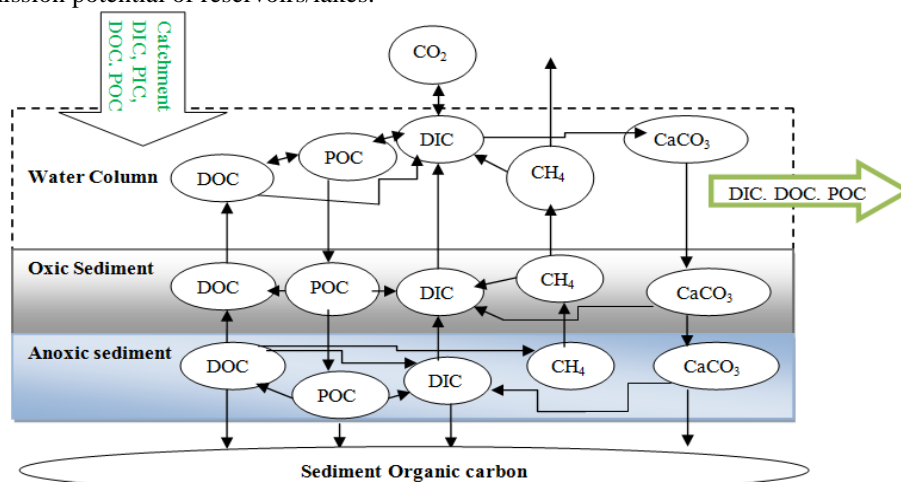


Figure 1: Mechanism of conversion of organic carbon into GHGs

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 (Figure1). 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 3: Physical, chemical and biochemical method for labile organic carbon analysis

Name of method	Types of carbon	Principle	Fractions	Advantages (+) disadvantages (-)	Ref.
(A) Physical					
Size fractionation	POC	Particles unable to pass through wet sieving	TOC in size fraction >53 μm	<ul style="list-style-type: none"> • Easy performance (+) • Inadequate knowledge about properties and function (-) • Possibility to involve inert charcoal (-) • Only quality characterization (-) 	[58, 59]
Densitometric separation	Light fraction	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Easy performance (+) • Only quality characterization (-) 	[60]
			Light : <1.6 g/ml Occluded I : 1.6 g/ml	<ul style="list-style-type: none"> • More finely distinguished fractions 	[61]

		(NaPT), • Size of the fractions determined by TOC	(+ultrasonic dispersion) Occluded II: 1.6-1.8 g/ml Occluded III : 1.8-2.0 g/ml Dense : >2.0 g/ml	(+) • Only quality characterization (-)	
Dissolved organic matter (DOM)	DOC	TOC dissolved in soil solution	TOC in field moist soil sample after centrifugation by 16,000 g for 30 min. at 4 °C (large stone removed & aggregates broken by hand).	• Evaluation of the actual level of organic matter scattered in water solution (+) • Easy performance (+) • Only quality characterization (-)	[62]
Water extractable organic matter (WEOM)	Hot water soluble carbon	Hot water-extractable carbon	TOC in extract: 60 min. gentle boiling in distilled water (DW). TOC in extract: 16 h at 80 °C shaking in DW.	• Easy performance with good results (+) • Only quality characterization (-) • Does not involve complete labile fraction (-)	[63-65]
	Cold water soluble carbon	Cold water-extractable carbon	TOC in extract: 30 min at 20 °C shaking in DW.	• Easy performance (+) • Bad result (-)	[64]
	water soluble carbon	water soluble carbon	TOC in extract: 1 h at 20 °C shaking in DW, followed by centrifugation and filtration.	• Only quality characterization (-)	[66]
(B) Chemical					
Oxidation	• Carbon oxidized with potassium	Use KMnO ₄ as oxidizing agent	• Fraction I = C oxidized by 333 mM KMnO ₄ • Fraction II = TOC – fraction I	• Easy performance (+) • Only quality characterization (-)	[67-70]
	• Modified Walkley-Black method	C oxidized by 0.167 M K ₂ Cr ₂ O ₇	• Fraction 1 : 6 M H ₂ SO ₄ • Fraction 2 : 9 M H ₂ SO ₄ - F1 • Fraction 3 : 12 M H ₂ SO ₄ - F2 • Fraction 4 : TOC - F3	• Characterises both quality and quantity (+) • Widely accepted and not expensive (+) • Sensitive distinction of fractions (+) • Recovery value of OC carbon is 76% (+) • Precision of measurement is good (+) • Poor reproducibility of results (-)	[69, 71-73]
	Sequential oxidation	C oxidised with K ₂ Cr ₂ O ₇ + H ₂ SO ₄ mixture (45 min. at 125 °C) and retitration with 0.1 M Fe ²⁺	• Fraction 1 =0.017 M K ₂ Cr ₂ O ₇ + 2.25 M H ₂ SO ₄ • Fraction 2 =0.0333 M K ₂ Cr ₂ O ₇ + 4.50 M H ₂ SO ₄ • Fraction 3 =0.0500 M K ₂ Cr ₂ O ₇ + 6.75 M H ₂ SO ₄ • Fraction 4 =0.067 M K ₂ Cr ₂ O ₇ + 9.00 M H ₂ SO ₄	• Characterises both quality and quantity (+) • Good result (+) • Risky chemical is in small concentration (+) • Less sensitive feature of fraction (-)	[50]
Dissolved organic matter (DOM)	DOC	• K ₂ SO ₄	• Extraction of soil (0-6 h) with either distilled water or 0.5 M K ₂ SO ₄ (20 °C at 4 °C or in the presence of an inhibitor of microbial activity (HgCl ₂ and Na-azide)).	• Characterises both quality and quantity (+) • Good result (+)	[74]
Acid hydrolysis	Decomposable and resistant plant material (lignin and fat resin)	• TOC in H ₂ SO ₄ hydrolysate	• Labile pool I : 2.5 M H ₂ SO ₄ (30 min. at 105 °C) • Labile pool I : 13 M H ₂ SO ₄ (20 °C overnight next dilution to 1 M H ₂ SO ₄ , 3h at 105 °C)- LP I • Recalcitrant pool : TOC – LP II	• Very sensitively distinguish fractions (+) • Suitable to various substrates (+) • Characterises both quality and quantity (+)	[75, 76]

		<ul style="list-style-type: none"> • TOC in HCl hydrolysate 	<ul style="list-style-type: none"> • Hydrolysable in 1 M HCl • Hydrolysable in 6 M HCl 	<ul style="list-style-type: none"> • Simplest (+) • Most reproducible method (+) • Used for Easily degradable compounds like amino acids, amino sugar, soluble carbohydrates, and microbial biomass (+) 	[77-84]
(C) Biochemical					
Micro-organism activity	<ul style="list-style-type: none"> • Microbial biomass carbon 	C difference between fumigated and non-fumigated soil sample	<ul style="list-style-type: none"> • Fumigation with chloroform for 24 h, followed by extraction with 0.5 M K₂SO₄ for 2 h (shaking) • C determined by chemical oxygen demand (COD) or TOC 	<ul style="list-style-type: none"> • Good concept for determination of micro-organism amount (+) • No estimation of enzymes activity (-) 	[65, 85]
	<ul style="list-style-type: none"> • Basal soil respiration 	CO ₂ evolved from soil during incubation	<ul style="list-style-type: none"> • 20 h incubation at 28⁰C • Evolved CO₂ estimated 	<ul style="list-style-type: none"> • True mineralisable organic matter(+) • Takes short time(+) • Characterises both quality and quantity (+) • Only most labile compound are mineralized (-) 	[86]
Micro-organism activity & substrate quality	Mineralizable C	CO ₂ evolved from soil during incubation	<ul style="list-style-type: none"> • 24 day incubation at 25⁰C • CO₂ evolved trapped in 1.0 M NaOH • Surplus of alkali titrated with 1.0 M HCl 	<ul style="list-style-type: none"> • True mineralization organic matter(+) • Characterises both quality and quantity (+) • Takes long time (-) 	[87]
	Mineralizable N	NH ₄ evolved during anaerobic incubation	<ul style="list-style-type: none"> • 7 days incubation at 40⁰C • NH₄⁺ is determined as total organic nitrogen (TON) 	<ul style="list-style-type: none"> • Characterises both quality and quantity (+) • Only most labile compound are mineralized (-) 	[88]
	BOD and K ₁	Biochemical oxygen demand and reaction rate constant	<ul style="list-style-type: none"> • % day BOD₅ determined manometrically • Calculation of reaction rate constant 	<ul style="list-style-type: none"> • Easy performance (+) • Characterises both quality and quantity (+) • Cost retentively high (-) 	[89]
(D) Instrumental					
CHN analyzer	TOC	Removal of inorganic carbonates and de-gassing of CO ₂	<ul style="list-style-type: none"> • Decarbonation : 20 cm³ of 0.5 M HCl to 10 g of sediment in a petri dish • leaving for 30 min • Oven at 105⁰C for 1 h. • 2 mg of dried at 105⁰C was weighed in a tin capsule using a Leco 650 microbalance 	<ul style="list-style-type: none"> • Coefficient of variation (CV): 3 % (+) • Loss of OC due to decarbonation (-) • Formation of hygroscopic chloride (-) 	[90]
Carbon analyzer (Dry combustion)	DOC, DIC and POC	OC is oxidized in a furnace followed by direct determination of the evolved CO ₂ .	-----	<ul style="list-style-type: none"> • Accurate and quick result (+) • Expensive instrument (-) • Consumables, need pure O₂ gas (-) 	[91]

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

1. Viollier, E., Rabouille, C., Apitz, S.E., Breuerd, E., Chaillou, G., Dedieub, K., Furukawaf, Y., Grenz, C., Hall, P., Janssen, F., *Journal of Experimental Marine Biology and Ecology*. 285 (2003) 5-31.
2. Middelburg, J.J., Nieuwenhuize, J., Van-Breugel, P., *Marine Chemistry*. 65 (1999) 245- 252.
3. Zegouagh, Y., Derenne, S., Largeau, C., Bertrand, P., Sicre, M.A., Saliot, A., Rousseau, B., *Organic Geochemistry*. 30 (1999) 101-117.
4. Fabiano, M., Danovaro, R., Frascchetti, S., *Continental Shelf Research*. 15 (1995) 1453-1469.
5. Dellanno, A., Mei, M.L., Pusceddu, A., Danovaro, R., *Marine Pollution Bulletin*. 44 (2002) 611-622.
6. Keil, R.G., Montlucon, D.B., Prahl, F.G., Hedges, J.I., *Nature*. 370 (1994) 549-552.
7. Dell'Anno, A., Fabiano, M., Mei, M.L., Danovaro, R., *Marine Ecology Progress Series*. 196 (2000) 15-23.
8. Fichez, R., *Oceanol Acta*. 14 (1991) 369-377.
9. Danovaro, R., Fabiano, M., Della, C.N., *Deep Sea Research Part I*. 40 (1993) 953-965.
10. Fabiano, M., Danovaro, R., Frascchetti, S., *Continental Shelf Research*. 15 (1995) 1453-1469.
11. Tselepidis, A., Polychronaki, T., Marrale, D., Akoumianaki, I., Dell'Anno, A., Pusceddu, A., Danovaro, R., *Progress in Oceanography*. 46 (2000) 311-344.
12. Buscail, R., Pocklington, R., Germain, C., *Continental Shelf Research*. 15 (1995) 843-869.
13. George, J.D., *Limnology Oceanography*. 9 (1964) 453-455.
14. Mayer L.M., Schick L.L., Sawyer T., Plante C.J., Jumars P.A., Self R.L., *Limnology Oceanography*. 40 (1995) 511.
15. Dauwe, B., Middelburg, J.J., Van Rijswijk, P., Sinke, J., Herman, P.M.J., Heip, C.H.R., *Journal of Marine Research*. 57 (1999) 109.
16. Parton, W.P., Schimel, D.S., Cole, C.V., Ojima, D.S., *Soil Science Society of America Journal*. 51 (1987) 1173.
17. Dalal, R.C., Chan, K.Y., *Australian Journal of Soil Res.* 39 (2002) 435-464.
18. Baldock, J.A., Skjemstad, J.O., Soil organic carbon/soil organic matter, In: 'Soil Analysis: An Interpretation Manual', Melbourne; (1999) 159-170.
19. Cambardella, C.A., Elliott, E.T., *Soil Science Society of America Journal*. 56 (1992) 777-783.
20. Franzluebbers, A.J. Stuedemann, J.A., *Soil Science Society of America Journal*. 67 (2003) 132-138.
21. Drees, L.R., Wilding, L.P., Smeck, N.E., Senkayi, A. L., Silica in soils: quartz and disordered silica polymorphs. In: 'Mineral in Soil Environments', 2nd edn. (Eds J. B. Dixon and S. B. Weed), Madison; (1989) 471-552.
22. Parr, J.F., Sullivan, L.A., *Soil Biology & Biochemistry*. 37 (2005) 117-124.
23. Cerling, T.E., *Earth and Planetary Science Letters*. 71 (1984) 229-240.
24. Dalal, R.C., Mayer R.J., *Australian Journal of Soil Research*. 24 (1986) 265-279.
25. Knowles, T.A., Singh, B., *Australian Journal of Soil Research*. 41 (2003) 889-903.
26. Nelson, D.W., Sommers, L.E., *Soil Sci. Soc. Am. Madison, Wisconsin, USA* (1996)5.
27. Maestre, S.E., Mora, J., Hernandez, V., Todoli, J.L., *Analytical Chemistry*. 75 (2003) 111.
28. Barcelona, M.J., *Ground Water*. 22 (1984) 18.
29. Urbansky, E.T., *Journal of Environmental Monitoring*. 3 (2001) 102.
30. Dell'Abate, M.T., Canali, S., Trinchera, A., Benedetti, A., Sequi, P., *Nutrient Cycling in Agroecosystems*. 51 (1998) 217.
31. Soon, Y.K., Abboud, S., *Communications in Soil Science and Plant Analysis*. 22 (1991) 943.
32. Nieuwenhuize, J., Maas, Y.E.M., Middelburg, J.J., *Marine Chemistry*. 45 (1994) 217
33. Benner, R., Vonbodungen, B., Farrington, J., Hedges, J., Lee, C., Mantoura, F., Suzuki, Y., Williams, P.M., *Marine Chemistry*. 41 (1993) 5.
34. Sharp, J.H., Peltzer, E.T., Alperin, M.J., Cauwet, G., Farrington, J.W., Fry, B., Karl, D.M., Martin, J.H., Spitzky, A., Tugrul, S., Carlson, C.A., *Marine Chemistry*. 41 (1993) 37.
35. Kimble, J.M., Lal, R., Follett, R.F., Lal, J.M.K.R., Follett, R.F., Stewart, B.A., Assessment Methods for Soil Carbon, *Lewis Publishers*, Boca Raton, Florida, USA. (2001) 553-575.
36. Froelich, P.N., *Limnology Oceanography*. 25 (1980) 564.
37. Caughey M.E., Barcelona M.J., Powell R.M., Cahill R.A., Gron C., Lawrenz D., Meschi P.L., *Envir. Geol.* 26 (1995) 211.
38. Jolivet, C., Arrouays, D., Bernoux, M., *Commun. Soil Sci. Plant Anal.* 29 (1998) 2227.
39. Rosell, R.A., Gasparoni, J.C., Lal, J.M.K.R., Follett, R.F., Stewart, B.A., Assessment Methods for Soil Carbon, *Lewis Publishers*, Boca Raton, Florida, USA, (2001) 311-322.

40. Poirier N., Sohi S.P., Gaunt J.L., Mahieu N., Randall E.W., Powlson D.S., Evershed R.P., *Org Geochem.* 36 (2005) 1174.
41. Van Hees, P.A.W., Jones, D.L., Finlay, R., Godbold, D.L., *Soil Biology & Biochemistry.* 37 (2005)1-13.
42. Van Hees, P.A.W., Johansson, E., Jones, D.L., *Plant and Soil.* 310 (2008) 11-23.
43. Bengtson, P., Bengtsson, G., *Ecology Letters.* 10 (2007) 783-790.
44. Boddy, E., Hill, P.W., Farrar, J., Jones, D.L., *Soil Biology & Biochemistry.* 39 (2007) 827-835.
45. Oburger, E., Jones, D.L., *Soil Biology & Biochemistry.* 41 (2009) 1951-1956.
46. Capriel, P., *Eur J. Soil Sci.* 48 (1997) 457-462.
47. Balaria, A., Johnson, C.E., Xu, Z.H., *Soil Science Society of America Journal.* 73 (2009) 812-821.
48. Chan, K.Y., Bowman, A., Oates, A., *Soil Science.* 166 (2001) 61-67.
49. Walkley, A., Black, I.A., *Soil Science.* 37 (1934) 29-38.
50. Strosser, E., Soil organic matter evaluation system based on hydrophilic fractionation and characterization of the fractions with differential thermic analysis. *Dipoloma thesis*, ZF JU, ceske Budejovice (in Czech). (2008)49-60.
51. Rovira, P., Vallejo, V.R., *Communication in Soil Science Plant Analysis.* 31 (2000) 81-100.
52. Shirato, Y., Yokozawa, M., *Soil Biology and Biochemistry.* 38 (2006) 812-816.
53. Plante, A.F., Conant, R.T., Paul, E.A., Paustian, K., Six, J., *European Journal of Soil Science.* 57 (2006) 456-467.
54. Carter, M.R., *Soil Tillage Research.* 7 (1986) 29-40.
55. Sparling, G.P., *Aust J Soil Res.* 30 (1992) 195-207.
56. Adamczyk, B., Kitunen, V., Smolander, A., *Soil Biology and Biochemistry.* 41 (2009) 2085- 2093.
57. Kolar, L., Klimes, F., Ledvina, R., Kuzel, S., *Plant Soil Environ.* 49(2003) 8-11.
58. Gregorich E.G., Beare M.H., Mckim U.F., Skjemstad J.O., *Soil Science Society of America Journal.* 70 (2006) 975.
59. Baldock, J.A., Composition and cycling of organic carbon in soil. In Marschner P, Rengel Z (eds.): Nutrient cycling in terrestrial ecosystems, London, Springer, (2007) 1-35.
60. Alvarez, C.R. Alvarez, R., Grigera, S., Lavado, R.S., *Soil Biology and Biochemistry.* 30 (1998) 767-773.
61. Rovira, P. Vallejo, V.R., *Soil Biology and Biochemistry.* 35 (2003) 245-261.
62. Giesler, R., Lundstrom, U., *Soil Science Society of America Journal.* 57 (1993) 1283-1288.
63. Korschens, M., Schulz, E., Behm, R., *Zbl Mikrobiol.* 145 (1990) 305-311.
64. Ghani, A., Dexter, M., Perrott, K.W., *Soil Biology and Biochemistry.* 35 (2003) 1231-1243.
65. Uchida, Y., Nishimura, S., Akiyama, H., *Agriculture, Ecosystems and Environment.* 156 (2012) 116- 122.
66. Tirol-Padre, A., Ladha, J.K., *Soil Science Society of America Journal.* 68 (2004) 969-978.
67. Baham, J., Sposito, G., *Journal of environmental quality.* 12 (1983) 96-100.
68. Blair, N., *Soil and Tillage Research.* 91 (2006) 39-47.
69. Zhao, X., Cheng, J., *Procedia Environmental Sciences.* 10 (2011) 1768 - 1773.
70. Culman S.W., Snapp S.S., Freeman M.A., Schipanski M.E., Beniston J., Lal R., Drinkwater L.E., Franzluebbers A.J., Glover J.D., Grandy A.S., Lee J., Six J., Maul J.E., Mirksy S.B., Spargo J.T., *Soil Sci. Soc. Am. J.* 76 (2012) 494.
71. Wang, X.J., Smethurst, P.J., Herbert, A.M., *Australian Journal of Soil Research.* 34(1996) 545-553.
72. Leong, L.S., Tanner, P.A., *Marine Pollution Bulletin.* 38 (1999) 875-879.
73. Chan, K.Y., Bowman, A., Oates, A., *Soil Sci.* 166 (2001) 61-67.
74. Rousk, J., Jones, D.L., *Soil Biology & Biochemistry.* 42 (2010) 2331-2335.
75. Rovira, P., Vallejo, V.R., *Communication in Soil Science and Plant Analysis.* 31(2000) 81-100.
76. Shirato, Y., Yokozawa, M., *Soil Biol Biochem.* 38 (2006) 812-816.
77. Leavitt, S.W., Follett, R.F., Paul E.A., *Radiocarbon.* 38 (1996) 231-239.
78. Hu, S., Coleman, D.C., Carroll, C.R., Hendrix, P.F., Beare, M.H., *Agriculture Ecosystems and Environment.* 65 (1997) 69.
79. Paul, E.A., Morris, S.J., Bohm, S., The determination of soil C pool sizes and turnover rates: biophysical fractionation and tracers, *Assessment Methods for Soil Carbon.* New York: Lewis Publishers. (2001)193-206.
80. Paul, E.A., Morris, S.J., Conant, R.T., Plante, A.F., *Soil Science Society of America Journal.* 70 (2006) 1023-1035.
81. Collins, H.P., Elliott, E.T., Paustian, K., Bundy, L.G., Dick, W.A., Huggins, D.R., Smucker, A.J.M., Paul, E.A., *Soil Biology & Biochemistry.* 32 (2000) 157-168.
82. Rovira, P., Vallejo, V.R., *Geoderma.* 107 (2002) 109-141.
83. Hobbie, E.A., Gregg, J., Olszyk, D.M., Rygielwicz, P.T., *Global Change Biology.* 8 (2002) 1072-1084.
84. Silveira, M.L., Comerford, N.B., Reddy, K.R., Cooper, W.T., El-Rifai, H., *Geoderma.* 144 (2008) 405-414.
85. Vance, E.D., Brookes, P.C., Jenkinson, D.S., *Soil Biology and Biochemistry.* 19 (1987) 703-707.
86. Novak, B., Apfelthaler, R., Rostl, V., *Rostl Vyr.* 10 (1964) 145-150.
87. Majumder, B., Mandal, B., Bandyopadhyay, P.K., Chandhury, J., *Plant Soil.* 297 (2007) 53-67.
88. Keeney, D.R., Bremner, J.M., *Argon J.* 58 (1996) 498-503.
89. Kolar, L., Klimes, F., Ledvina, R., Kuzel, S., *Plant Soil and Environment.* 49 (2003) 8-11.
90. Chang, F.Y., Kao, S.J., Liu, K.K., *Acta Oceanographica Taiwanica.* 27 (1991) 140-150.
91. Pansu, M., Gautheyrou, J., Loyer, J., *Soil Analysis-Sampling, Instrumentation and Quality Control.* Paris. (2001) 512.