

COMPARATIVE STUDY OF TITIMETRIC METHODS FOR DETERMINATION OF ORGANIC CARBON IN SOILS, COMPOST AND SLUDGE

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Abstract

The aim of this study was to evaluate different titrimetric methods of organic carbon determination (OC), using reference soils, compost and sludge as standards, as well as to clarify the effects of the phosphoric acid and the efficiency of the indicators used in the titration. SOC content was determined by 10 wet oxidation methods (7 which are commonly used in soil testing laboratories and 3 methods modified by us). The agreement between certified and measured results suggested that SOC could be completely recovered by using wet digestion under tested method described (except Walkley Black modified method). Organic carbon contents from the Walkley Black modified method were higher in all investigated samples than those obtained by the other titrimetric methods. The most significant increase is observed in the standards 3, 6 and 7, i.e. the standards with the highest content of organic matter. The Tube digestion method and Tjurin method (1500C with phosphoric acid added before titration) are very effective for soil samples up to 2 % of organic carbon. The Tube digestion method for organic carbon determination seem to be the most suitable methods, for soil samples, compost and sludge with high organic matter content. The indicators do not significantly affect the quantification of organic carbon in most studied samples. The determined values for all standard samples with indicator diphenylamine are little bit than those determined using the N-phenylanthranilic acid and o-phenanthroline. N-phenylanthranilic acid can be used with H₃PO₄ addition before titration. Ferroin can be as effective as barium diphenylamine sulfonate if soil is filtered or centrifuged to get clear solution after the addition of water in the procedure, but this process involves a lot of time, hence barium diphenylamine sulfonate and N-phenylanthranilic acid, as indicators, are recommended.

Key words: *titrimetric methods, organic carbon determination, certified samples*

INTRODUCTION

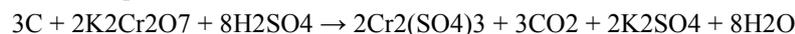
Soil humus is traditionally considered a continuous source of slow-release nutrients and a reservoir of organic colloids playing a major role in the regulating processes of plant nutrition, toxic ion mobility, and the aggregation, structure and water-holding capacity of the soil (Shpedt et al., 2001). Humus content and composition are considered integral parameters of soil fertility and variable ecological processes, and a source of information for the early diagnosis of soil degradation (Arlauskas and Slepetiene, 1997; Ellerbrock et al., 1999). Soil organic matter (SOM) is usually classified into nonhumic and humic materials (Klavins, 1997). Humic material is only one portion, but it is a major portion of organic matter in most soils and waters. Humic materials are transformed SOM, having lost, during the humification process, both their morphological resemblance to the structures from which they were derived and their cell structure (Slepetiene and Butkute, 2003).

A great variety of methods exists for the estimation of both humus content and its fractional composition, which are used depending on researchers' tasks, available facilities, methodological conventionality and experience. A major part of the methods used for the quantitative determination of humus and its constituent parts is based on the evaluation of the amount of organic carbon (OC). Carbon in soil samples is converted to carbon dioxide which is measured directly or indirectly by different methods. Soil organic carbon is commonly measured by dry combustion at high temperatures in a furnace with the collection and detection of evolved CO₂ with automated analyzers, (Schumacher, 2002) or a wet chemical oxidation method followed by titration of the remaining dichromate with ferrous ammonium sulfate or photometric determination of Cr³⁺ (Ellerbrock et al., 1999). The dry combustion method is considered as the most precise and accurate procedure today but the high cost of dry combustion is major limitation to many laboratories (Konen et al., 2002).

The dichromate procedures are widely used for routine soil organic carbon determinations in laboratories where the purchase of an automated system can not be justified, because of the simplicity and rapidity of the method compared with dry combustion (Nelson and Sommers, 1975). These techniques can be divided into two phases, namely, sample extraction and sample quantitation. The extraction technique employed is essentially the same for all methods in the literature with variations existing only in the strength and combination of reagents used during extraction. Quantitation techniques associated with the wet chemistry determination of TOC either rely on titration, or by colorimetric techniques (Nelson and Sommers, 1982).

The first stage is organic matter oxidation by heating with a potassium dichromate solution in sulfuric acid (Ponomariova and Plotnikova, 1980). The second stage is based on the estimation of the amount of potassium dichromate used for OC oxidation, evaluated by titration or photometrically (Ponomariova and Plotnikova, 1980);

Nikitin, 1999, Filcheva and Tsadilas, 2002; Spiegel et al., 2007). Oxidation occurs according to the following reaction equation:



orange colour green colour

After the reaction, the excess $Cr_2O_7^{2-}$ is titrated with $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, and the $Cr_2O_7^{2-}$ reduced during the reaction with soil is assumed to be equivalent to the organic C present in the sample. It must be emphasized that all methods based on determination of $Cr_2O_7^{2-}$ remaining or Cr^{3+} formed assume that C in soil organic matter has an average valence of zero.

The most frequently used methods are Walkley and Black (1934), Walkley-Black modified - with external heating, Kalembasa and Jenkinson (1973), Tjurin method (Ponomareva and Plotnikova, 1975) and Tjurin method modified (Kononova, 1966; Filcheva et al., 1996; Filcheva and Tsadilas, 2002). The Walkley Black-Black methods have been used and sometimes are still in use in the Western European countries as well as in USA (FAO), the Tjurin methods mainly in the Central and East European Countries.

Schollenberger (1927) used concentrated H_2SO_4 (18 M or 36 N) as the solvent for $K_2Cr_2O_7$. Although most dichromate oxidation procedures described since the original Schollenberger method have involved chromic acid solutions or mixtures of concentrated H_2SO_4 and aqueous $K_2Cr_2O_7$ solutions, Tinsley (1950) and Kalembasa and Jenkinson (1973) proposed addition of H_3PO_4 to the chromic acid mixture used to oxidize organic C compounds. There is no evidence, however, to suggest that oxidation mixtures containing H_3PO_4 are more efficient in oxidizing organic matter than $K_2Cr_2O_7$ - H_2SO_4 mixtures. The oxidizing mixtures used in most published methods are between 0.16 and 0.35 M in $K_2Cr_2O_7$ and 7.5 and 12.5 M (15 and 25 N) in H_2SO_4 . However, Tinsley (1950), Nelson and Sommers (1975) and Yeomans and Bremner (1988) used an aqueous H_2SO_4 -water mixture that was 0.145 M (0.87 N) in $K_2Cr_2O_7$.

Rapid dichromate oxidation techniques have employed heating times and temperatures that vary from no external heat to extensive boiling of chromic acid mixtures. Schollenberger (1927) suggested that the soil- H_2SO_4 - $K_2Cr_2O_7$ mixture be heated in a Pyrex test tube over a flame until the solution temperature reached $175^\circ C$ at which time heating was discontinued. Later investigators realized that the time and temperature of heating were critical and must be standardized to insure that a constant proportion of soil organic matter was oxidized and that a consistent amount of dichromate was thermally decomposed during the digestion. Jackson (1958) suggested that the soil-chromic acid mixtures be heated for defined periods (5-10 min) in test tubes submerged in H_2SO_4 or oil baths maintained at prescribed temperatures (140 - $170^\circ C$).

Walkley and Black (1934), however, proposed that the heat of dilution of H_2SO_4 ($120^\circ C$) was satisfactory for oxidizing 75% of the organic C in soils and that a correction factor could be used to account for incomplete digestion. Several investigators have found that an extended period of heating is required to obtain quantitative oxidation of soil organic C by chromic acid (Anne, 1945; Tinsley, 1950; Mcbius, 1960; Kalembasa and Jenkinson, 1973; Heanes, 1984). High digestion temperatures ($>145^\circ C$) lead to thermal decomposition of dichromate and resultant high blank values (Tinsley, 1950; Metson et al., 1979; Heanes, 1984). Clay minerals have been reported to catalyze the thermal decomposition of $Cr_2O_7^{2-}$ (Walkley, 1947) but a recent study has shown little thermal decomposition when high clay soils free of organic matter were heated with $K_2Cr_2O_7$ - H_2SO_4 for 60 min at 125 or $145^\circ C$ (Heanes, 1984). Digestion temperature is normally regulated by the ratio of water/ H_2SO_4 in the mixture and the temperature rises as water vapor is lost during heating. Tinsley (1950) proposed that cold finger condensers fitted on Erlenmeyer flasks be used to prevent loss of water during digestion, whereas other investigators have used Erlenmeyer flasks fitted for estimation of organic C all employing 100-ml tubes. Heanes (1984), Yeomans and Bremner (1988), and Soon and Abboud (1991) recommended heating for 30 min at $135^\circ C$, $170^\circ C$, and $155^\circ C$, respectively. Yeomans and Bremner (1988) and Soon and Abboud (1991) specify the same digestion reagents as Nelson and Sommers (1975), i.e., 5 ml of 0.167 M $K_2Cr_2O_7$ and 7.5 ml of concentrated H_2SO_4 , whereas Heanes (1984) recommends 10 ml of 0.167 M $K_2Cr_2O_7$ and 20 ml of H_2SO_4 . Tube digestion procedures have been reported to yield organic C values that approximate those from dry and wet combustion techniques (Sparks, 1996).

The reduction of dichromate is also affected by the presence of other redox-active components of the soil. The presence of Fe^{2+} , Mn^{2+} , and Cl^- causes an overestimation of soil OC, since all three are oxidized during the digestion.

An additional problem is presented by the often low visibility of the color change during back titration. Indicators used are o-phenanthroline (green to reddish brown) or N-phenylanthranilic acid (dark violet-green to light green). Some workers prefer phenylanthranilic acid, but in clayey soils with high chroma, the color change may be difficult to observe. Both indicators require some experience before overtitration can be avoided.

Since all methods available have some limitations, the choice of assessment methodology is a critical decision for accurate quantification of SOC content.

The aim of this study was to evaluate different titrimetric methods of organic carbon determination (OC), as well as their comparability and compatibility, accuracy, precision, speed of determination and convenience, using reference soils, compost and sludge as standards. Additional investigations are conducted to clarify the effects of the phosphoric acid and the efficiency of the indicators used in the titration.

MATERIALS AND METHODS

For comparison of methods were used 7 certified samples (soils, compost and sludge) containing total carbon (%) as follows: NCS DC 73386 – 0.32 ± 0.13 , ISE 859 – 11.4 ± 1.13 , ISE 952 – 1.66 ± 0.204 , and organic matter (%) as follows NCS DC 73386 – 9.1 ± 0.13 , NCS DC 85106a – 3.85 ± 0.13 , ISE 859 – 21.7 ± 1.0 , ISE 952 – 9.17 ± 1.005 , ISE 952 – 14.0 ± 0.093 , MARSE P205 – 42.4 ± 0.85 и MARSE P206 – 54.4 ± 0.76 . The main characteristics of the certified samples and the parameters that affect the organic carbon content, are presented in Table 1.

Table 1. The main characteristics of the certified samples

№	Standard	Certified material	Organic carbon, %	Organic matter, %	Total Fe, mg/kg	Total Mn, mg/kg	Cl, mg/kg (soluble in water)	pH
1	NCS DC 73386	Soil	0.32 ± 0.13	9.1 ± 0.13		1360 ± 71	76	-
2	NCS DC 85106a	Soil		3.85 ± 0.13	258 ± 30 (available)	88 ± 7 (available)	0.16 ± 0.02	6.80 ± 0.08
3	ISE 859	Chanel sludge	11.4 ± 1.13	21.7 ± 1.0	42700 ± 2170	909 ± 44	140	7.0 ± 0.151
4	ISE 952	Clay	2.13 ± 0.292	9.17 ± 1.005	61300 ± 2240	1520 ± 77	42.0	5.96 ± 0.207
5	ISE 998	Organic Ferrasol	1.66 ± 0.204	14.0 ± 0.93	90200 ± 3360	174 ± 22.1	17.0	3.88 ± 0.163
6	MARSE P205	Compost		42.4 ± 0.85		10600 ± 900		
7	MARSE P206	Sweage Sludge		54.4 ± 0.76	52400 ± 440	615 ± 18.6		

Note - the organic carbon content is determined titrimetrically, organic matter content by loss on ignition method, with the exception of sample 2 (calculation from organic carbon in organic matter)

Organic carbon in the certified samples was determined by 10 wet oxidation methods (7 which are commonly used in soil testing laboratories described below (methods of Tjurin (Ponomareva and Plotnikova (1975) version), Tjurin modified method (Konova (1966) version), Kalembasa and Jenkinson (1973), Tinsley (1950), Tube digestion method, Walkley and Black method (1934) and Walkley - Black modified method) and 3 methods modified by us), all of the methods being based on dichromate consumption. We modified the Turin method (at 120 and 1500C) and Tube digestion procedure by addition of phosphoric acid before titration and tested this modification (Table 2).

Tjurin method (Ponomareva and Plotnikova (1975) version of the Tyurin method was used): Depending on the humus content samples of 0.05-1.0 g are taken from the samples and after a dichromate digestion with 10 ml 0.4 N oxidizing solution ($K_2Cr_2O_4$: H_2SO_4 -1:1) at 120°C for 45 min in presence of catalyser Ag_2SO_4 , the consumed oxidizer (oxidizing agent) is determined by titration with $Fe(NH_4)_2(SO_4) \cdot 6H_2O$ using phenylanthranilic acid as an indicator.

Tyurin method (Kononova's version (1966) of the Tyurin method was used): Appropriate quantity of soil sample was weighed into an Erlenmeyer flask and after a dichromate digestion with 10 ml 0.4 N oxidizing solution ($K_2Cr_2O_4$: H_2SO_4 -1:1) at 150 °C for 20 min in presence of catalyser Ag_2SO_4 , the consumed oxidizer (oxidizing agent) is determined by titration with $Fe(NH_4)_2(SO_4) \cdot 6H_2O$ using phenylanthranilic acid as an indicator.

Tube digestion method (Sparks, 1996): An amount of sample estimated to contain about 8 mg (usually 100-500 mg) of organic carbon was weighed into a clean, dry digestion tube and treated with 5 ml of 0.167 M (1.0 N) $K_2Cr_2O_7$ solution and 7.5 ml of concentrated H_2SO_4 . The tube was placed in the digestion block preheated to

150°C for exactly 30 min. The digestion tube was removed from the block and the samples cooled for 30 min at room temperature. The contents of the tube were quantitatively transferred to a 125-ml Erlenmeyer flask and titrated with 0.2 N ferrous ammonium sulfate solution using 0.2 ml of the N-phenylanthranilic acid solution as the indicator.

Tinsley (1950) An amount of sample estimated to contain about 10 mg. of organic carbon was weighed into a 500 ml, conical flask and treated with 25 ml of 0.4 N K₂Cr₂O₇, 25 ml of concentrated H₂SO₄, and 12.5 ml of concentrated H₃PO₄. The flask was fitted with a 'cold finger' condenser and was boiled gently on a hot plate for 2 hours. The mixture was cooled, treated with 100 ml of water, and residual dichromate was determined by titration with 0.2 N ferrous ammonium sulphate (Fe(NH₄)₂(SO₄)₂·6H₂O) using barium diphenylamine sulphate solution (5 ml) as indicator.

Kalembasa and Jenkinson (1973): The soil, in a 250-ml Quickfit flask fitted with a Liebig condenser, was refluxed for 20 min with 20 ml of 0.5 N-potassium dichromate solution and 30 ml of a mixture of sulphuric and phosphoric acids (5 parts by volume of concentrated sulphuric acid, and 1 part of phosphoric acid). The soil sample must not contain more than 18 mg organic carbon; the preferred range is 5 to 15 mg. After cooling, the condenser was rinsed with 10 to 20 ml of water, 5 drops of indicator added (N-phenylanthranilic acid) and the suspension titrated with 0.2 N-ferrous ammonium sulphate.

Walkley -Black (1934) method: An amount of sample estimated to contain about 10 mg. of organic carbon was weighed into a 500 ml conical flask and treated with 10 ml of N K₂Cr₂O₇ and 20 ml of concentrated H₂SO₄, the latter being added rapidly from a fast-delivery burette. The flask was immediately swirled vigorously for 1 minute, allowed to stand on a sheet of asbestos for 30 minutes, and treated with water (200 ml) and concentrated H₃PO₄ (10 ml). The residual dichromate was determined by titration with using o-phenanthroline (three to four drops) as indicator.

Walkley -Black modified: An amount of sample estimated to contain about 10 mg. of organic carbon was weighed into a 250 ml conical flask and treated with 10 ml of 1 N K₂Cr₂O₇ and 20 ml of concentrated H₂SO₄, the latter being added rapidly from a fast-delivery burette. The flask was heat on a hot plate until the temperature reaches 135°C. The mixture was to be brought to boiling point rapidly and then simmered gently for 20 minutes allowed to stand on a sheet of asbestos for 30 minutes, and treated with water (200 ml) and H₃PO₄ (10 ml).

Table 2. Characteristics of tested method

№	Method	T, 0C	Time, min	Reagents	Add reagent	Indicator
1	Tjurin (Ponomareva and Plotnikova version)	120	45	Ag ₂ SO ₄ 0.4 N oxidizing solution (K ₂ Cr ₂ O ₇ + H ₂ SO ₄ -1:1)	-	N-phenylanthranilic acid
2	Tjurin (Ponomareva and Plotnikova version) (modified by us)	120	45	Ag ₂ SO ₄ 0.4 N oxidizing solution (K ₂ Cr ₂ O ₇ + H ₂ SO ₄ -1:1)	10 ml 85% H ₃ PO ₄ , before tittation	N-phenylanthranilic acid
3	Tjurin (Kononova's version)	150	30	0.4 N oxidizing solution (K ₂ Cr ₂ O ₇ + H ₂ SO ₄ -1:1)	-	N-phenylanthranilic acid
4	Tjurin (Kononova's version) (modified by us)	150	30	0.4 N oxidizing solution (K ₂ Cr ₂ O ₇ + H ₂ SO ₄ -1:1)	10 ml 85% H ₃ PO ₄ , before tittation	N-phenylanthranilic acid
5	Tube digestion method	150	30	5 ml 1N K ₂ Cr ₂ O ₇ 7.5 ml H ₂ SO ₄	-	N-phenylanthranilic acid
6	Tube digestion method (modified by us)	150	30	5 ml 1N K ₂ Cr ₂ O ₇ 7.5 ml H ₂ SO ₄	10 ml 85% H ₃ PO ₄ , before	N-phenylanthranilic acid

					tittation	
7	Kalembasa and Jenkinson	150	20	20 ml 0.5 N K ₂ Cr ₂ O ₇ 30 ml of a mixture H ₂ SO ₄ and H ₃ PO ₄	-	N-phenylanthranilic acid
8	Tinsley	150	120	25 ml 0.4 N K ₂ Cr ₂ O ₇ 25 ml H ₂ SO ₄ , 12.5 ml H ₃ PO ₄	-	barium diphenylamine sulphonate
9	Walkley and Black	without heating	30	10 ml 1N K ₂ Cr ₂ O ₇ 20 ml H ₂ SO ₄	10 ml 85% H ₃ PO ₄ , before tittation	1.o- phenantroline 2.barium diphenylamine sulphonate 3.N-phenylanthranilic acid
10	Walkley and Black –modified	135	30	10 ml 1N K ₂ Cr ₂ O ₇ 20 ml H ₂ SO ₄	10 ml 85% H ₃ PO ₄ , before tittation	o- phenantroline

All determinations were done in triplicate. A comparison of the results obtained for the organic carbon by titrimetric methods was performed by regression analysis using the SPSS program of Windows.

3. RESULTS AND DISCUSSION

3.1. A comparison between the methods used for organic carbon determination

The results obtained for the certified samples by titrimetric methods are presented in Table 3. The results obtained by the titrimetric methods were in good agreement with certified values for most samples analyzed. As can be seen from the Table 3 the results obtained for the content of organic carbon in the sample by titrimetric methods are similar to the certified value except with Walkley Black modification method. In this case, the results significantly exceed the certified values.

Table 3. Effectiveness of titrimetric methods for the determination of organic carbon in certified samples

	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
Certified value	0.32±0.05		11.4±1.3	2.13±0.29	1.66±0.20		
Methods without phosphoric acid							
Tjurin 120	0.27±0.07	2.05±0.25	10.71±1.6	2.23±0.30	1.70±0.20	20.57±0.80	24.32±0.80
Tjurin 150	0.32±0.05	2.06±0.25	11.35±1.3	2.21±0.29	1.67±0.25	19.40±0.90	23.52±0.80
Tube	0.39±0.06	1.99±0.28	12.66±1.2	2.13±0.28	1.63±0.25	22.25±0.80	24.36±0.90
Methods with addition of phosphoric acid in the mixture for decomposition							
Tinsley	0.27±0.05	2.17±0.23	12.37±1.1	2.26±0.29	1.72±0.20	23.02±0.85	26.94±0.96
Kalembasa and Jenkinson	0.39±0.05	2.11±0.25	12.42±1.3	2.67±0.29	1.65±0.22	22.43±0.78	24.34±0.90
Methods of addition of phosphoric acid before titration							
Tjurin120	0.27±0.07	2.03±0.25	11.56±1.3	2.25±0.29	1.75±0.20	20.67±0.80	24.84±0.85
Tjurin 150	0.27±0.08	2.04±0.25	12.24±1.3	2.20±0.25	1.76±0.20	19.53±0.90	23.72±0.89
Tube 150	0.34±0.05	1.94±0.27	11.24±1.3	2.22±0.25	1.71±0.18	21.49±0.80	23.87±0.85
Walkley and Black	0.25±0.05	1.96±0.30	10.95±1.5	1.77±0.39	1.51±0.25	20.50±0.80	22.31±0.80
Walkley	1.53±0.10	3.59±0.35	33.54±1.8	5.45±0.49	3.23±0.20	40.19±1.20	61.7±1.90

Black							
Modified							

Titrimetric methods used in this study can be divided into three groups - methods without the use of phosphoric acid, method in which phosphoric acid is added into the reaction mixture and the methods in which phosphoric acid is applied before titration. The comparison between the titrimetric methods and influence of phosphoric acid for determining the organic carbon is presented in Figure 1.

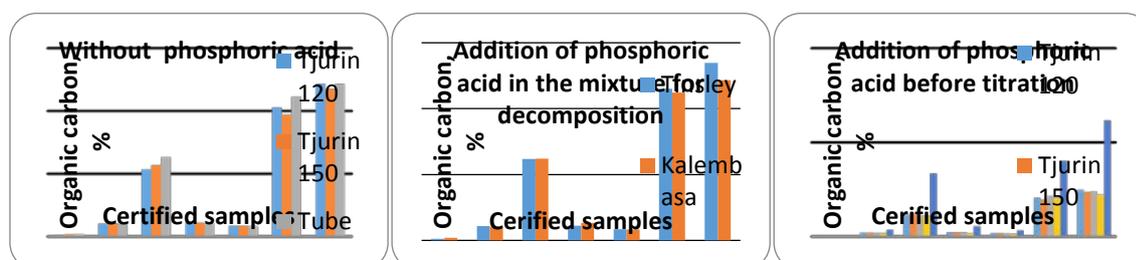


Figure 1. Influence of phosphoric acid for determination of organic carbon in titrimetric methods

When using titrimetric methods of analysis, the mechanisms of interaction on which the principle of the method itself is based are not to be overlooked. At the stage of 'oxidation of organic carbon' with dichromate mixture, part of the hexavalent chromium is reduced to trivalent chromium in the oxidation of the carbon precisely in the burning organic material which crosses from the zero to the fourth positive valence during the disintegration of the organic molecules. At the stage of 'titration of the remnant of the hexavalent chromium' in order to become trivalent, the role of a reducing agent is fulfilled by the divalent iron in the composition of Mohr's salt, which turns into trivalent. This is influenced by the added orthophosphoric acid which blocks the iron from the soil by turning it into a stable ferric phosphate. This facilitates the sharp colour transition because ferrous acid has the ability to turn from purple to colorless at the instant of the depletion of hexavalent chromium due to its transition to trivalent chromium (green colour). Tinsley (1950) and Kalembsa and Jenkinson (1973) add the phosphoric acid during the oxidation and Walkley Black adds it during titration but this does not affect the accuracy of the determination because this reagent is related to trivalent iron. The introduction of phosphoric acid influences the titrimetric methods. When added during the oxidation, the phosphoric acid promotes the release of calcium from the tightly bonded organic carbon in the composition of humin in more loamy, neutral or slightly alkaline (carbonate) soils, but when added during the titration it blocks the obstructive trivalent iron which is excessively present in the red acidified ferrasols. Obviously the reason for obtaining the good results in most standard samples is the addition of phosphoric acid before titration. The addition of H_3PO_4 to the digestive mix after the sample has cooled has been used to help eliminate interferences from the ferric (Fe^{3+}) iron that may be present in the sample although in most cases, this step is not necessary (Tiessen and Moir, 1993).

Walkley and Black method has been used as one of the standard methods to determine SOC (Sparks, 1996; Meersmans et al., 2009). The Walkley-Black procedure has been shown to lead to the incomplete oxidation of organic C and is particularly poor for digesting elemental C forms. Studies have shown that the recovery of organic C using the Walkley-Black procedure ranges from 60 to 86% with a mean recovery being 76% (Walkley and Black, 1934). As a result of the incomplete oxidation and in the absence of a site-specific correction factor, a correction factor of 1.33 is commonly applied to the results to adjust the organic C recovery. Dichromate methods that involve limited periods of heating (Walkley Black method) have been shown to yield good recoveries of organic C if appropriate correction factors are applied (Kalembsa and Jenkinson, 1973). Our results indicate that only in a standard 1 is requiring the use of a correction factor, while in the other samples it is not necessary.

To overcome the concern of incomplete digestion of the organic matter, the Walkley-Black procedure was modified to include extensive heating of the sample during sample digestion (Mebius, 1960). When external heat is applied during wet digestion of soil samples a complete digestion of SOC in the samples is achieved, and therefore no correction factor is needed (Schumacher, 2002). The addition of heat to the system leads to a complete digestion of the organic C in the sample; therefore, no correction factor is needed. Modern dichromate oxidation methods that involve an extended period of heating, often under reflux, (Tinsley, 1950; Mebius, 1960; Nelson and Sommers, 1975; Heanes, 1984; Yeomans and Bremner, 1988) give organic C values equivalent to those obtained by dry or wet combustion.

Organic carbon contents from the Walkley and Black modified method were higher in all investigated samples than those obtained by the other titrimetric methods (Table 2). The most significant increase is observed in the standards 3, 6 and 7, i.e. the standards with the highest content of organic matter. Obviously this increase is due of the used indicator (o-phenanthroline) in titration. The problem with the use of o-phenanthroline is that light may be absorbed by the soil suspended matter, thus leading to ambiguous transition and change in color of the end point are difficult to establish. In the method without heating obviously indicator does not affect (less suspended matter absorbed from the soil). In the method with heating (Walkley and Black modified method) the indicator affect and to reach the end point are spent much larger volumes from ferrous ammonium sulphate during the titration and we receive incorrect results.

Another possible reason is the clay content in the sample. Clays have been reported to cause decomposition of dichromate, leading to overestimated OC values (Metson et al. 1979), which was observed in sample 4 (Clay).

Table 3 shows the degree of extraction of organic carbon by titrimetric methods. As can be seen, the rate of recovery varies from 78.6-96.0% in Walkley and Black method, 84.1-105.6% in Tjurin method, 85.9 -108.5% in Tinsley method, 99.1 -125.4% in Kalembasa and Jenkinson method, 164-294% in Walkley and Black modified method. Results show that the degree of extraction of organic carbon obtained from different methods are comparable except with Walkley and Black modification method.

The basic principle of titrimetric methods, which are based on wet oxidation is the oxidation of soil organic matter with acidified potassium dichromate and the estimation of the amount of potassium dichromate used for organic matter oxidation. The variations between the methods existed in the concentration of $K_2Cr_2O_7$ solution, in the combination of reagents used, and in the oxidation conditions (heating time and temperature). Meersmans et al. (2009) suggested that note should be made on small differences in the methodology (the volume of potassium dichromate used or time/temperature of the oxidation reaction) which can have an important impact on the obtained SOC content. However, the evaluation of the results presented in this study showed that small differences in the methodology between the wet digestion procedures, used in this study, did not have an important influence on the obtained organic carbon content in the investigated soil samples, except by the Walkley and Black modified method.

Table 3. Mean values for the extraction of organic carbon (%) in the certified soil samples obtained by titrimetric method (n = 3).

	Standard 1	Standard 3	Standard 4	Standard 5
Methods without phosphoric acid				
Tjurin 120	85.69	93.99	104.92	102.68
Tjurin 150	100.54	99.54	103.88	100.83
Tube	122.84	111.01	100.18	98.48
Methods with addition of phosphoric acid in the mixture for decomposition				
Tinsley	85.87	108.48	105.98	103.86
Kalembasa and Jenkinson	121.95	108.96	125.41	99.10
Methods with addition of phosphoric acid before titration				
Tjurin120	84.16	101.32	105.60	105.47
Tjurin 150	85.69	107.37	103.31	106.14
Tube	106.72	98.59	104.20	102.74
Walkley -Black	78.63	96.01	83.09	90.74
Walkley- Black modified	164.53	294.19	255.87	194.41

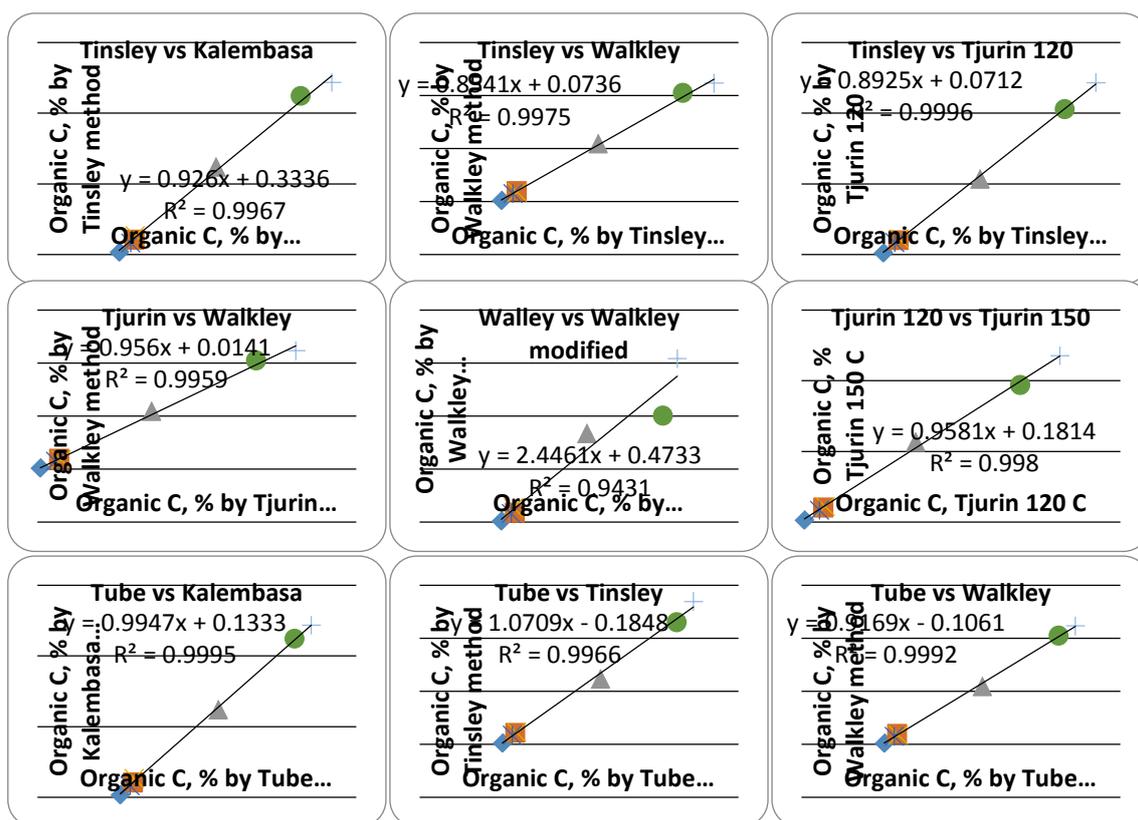


Figure 2. Relationship of soil organic carbon among different titrimetric method

For example, there are no significant differences between the results obtained by the methods of Turin. Raising the temperature from 120 to 150°C and reduce the time of heating the sample from 45 to 20 minutes did not affect the results obtained (Table 2). As a result of extensive heating, the tube digestion methods give complete recovery of organic C from certified samples (Tables 2 and 3). The Tube digestion method for organic carbon determination seem to be the most suitable methods, for soil samples, compost and sludge with low, moderately and high organic matter content in soils, compost and sludges. The major advantage of the tube digestion procedure is the decreased analysis time and reagents per sample (Bremner and Jenkinson, 1960; Kalembasa and Jenkinson, 1973; Nelson and Sommers, 1975).

The relationship between the titrimetric methods for determination of the organic carbon is presented in Figure 2.

The values of SOC content between the titrimetric methods are in a positive linear correlation (Figure 2). A comparison between organic carbon content, measured through titrimetric methods, from certified samples, resulted in a linear regression, demonstrating no significant differences between values of organic carbon obtained through different methods. The correlation coefficient equals 0.9, indicating a significant strong relationship between the variables (Figure 2). Chatterjee et al. (2009) reported that automated dry combustion is the only reliable method to determine SOC content in soils, but based on our results we could conclude that the titrimetric procedure gave results that are in very good agreement with the reference values and that they could give reliable results as well.

3.2. Effectiveness of indicators for organic carbon determination

In volumetric analysis, in which the substances react on equivalence bases, the point of equivalence (end point) is detected by suitable indicator. The best indicator is one which gives the clear and sharp end point (easily discernable at the exact equivalence point of the reacting substances). The most common indicators used are ortho-phenanthroline ferrous complex (commercially available as "Ferriol"), barium diphenylamine sulfonate, and N-phenylanthranilic acid (Nelson and Sommers, 1996). All these three indicators are used for such end point detection in organic matter determination in soil with little modification in almost various soil testing laboratories. The use of barium diphenylamine sulfonate is not common in soil testing laboratories. The excess $\text{Cr}_2\text{O}_7^{2-}$ is titrated with ferrous ammonium sulfate ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$) or ferrous sulfate (FeSO_4) until color change occurs in the sample. Color changes associated with these indicators are: (1) green to reddish brown for the orthophenanthroline ferrous complex, (2) purple/blue to green for the barium diphenylamine

sulfonate, and (3) dark violet-green to light green for the N-phenylanlhranilic acid. The primary concern with the manual titration technique is the low visibility or subtlety of color changes during titration.

Ortho-phenanthroline ferrous complex, barium diphenylamine sulfonate and N-phenylanlhranilic acid were the indicators, used for their effectiveness.

To find out the effectiveness of these indicators in giving easily discernable end point in the titration was conducted additional research with Walkley and Black method in which the original indicator was replaced by barium diphenylamine sulphonate and N-phenylanlhranilic acid.

The results of the organic carbon content of tested soils with the different indicators are given in the Fig.3

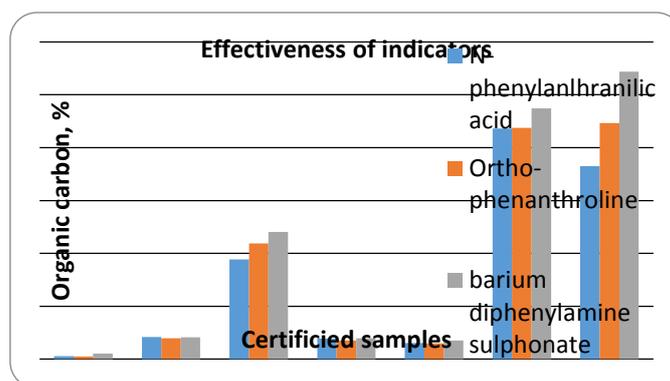


Figure.3. Effectiveness of indicators for organic carbon determination

Results shows that the indicators do not significantly affect the quantification of organic carbon in most studied samples. The determined values for all standard samples with indicator diphenylamine are little bit than those determined using the N-phenylanlhranilic acid and o-phenanthroline. Sometimes the desired end point is not clear, not because of the indicator, but due to some interfering substances in the solution (this interfering substance may already be present in the solution or formed during the reaction of the desired reagents). To overcome such interferences, this particular interfering effect is masked through appropriate means. In this analysis the clarity of end point is interfered through the formation of green colour of $\text{Fe}_2(\text{SO}_4)_3$, which is formed during the titration, orthophosphoric acid is used to mask such effect by the formation of FePO_4 . This interfering effect is only with diphenylamine and barium diphenylamine sulfonate indicators, as the end point is also green with these indicators. The use of orthophosphoric acid with ferroin indicator is optional (Allison, 1965).

It was suggested that the diphenylamine end point could be improved by addition of H_3PO_4 , or NaF, before titration (Walkley and Black, 1934), and these substances were widely used in dichromate titrations. Peech et al. (1947) established that barium diphenylamine sulfonate (diphenyl 4 sulfonic acid) in combination with H_3PO_4 was as effective and more stable compared with diphenylamine and has been used as an indicator in other procedures (Tinsley, 1950). Jackson (1958) recommended that o-phenanthroline be used as an indicator in $\text{Cr}_2\text{O}_7^{2-}$ titrations because the color change (formation of the complex with Fe^{3+}) occurs at higher oxidation-reduction potential compared with diphenylamine. A mixture of o-phenanthroline and H_3PO_4 is normally used to give a good end point; however, the indicator has been successfully used without H_3PO_4 addition. A problem with o-phenanthroline is that the indicator tends to be absorbed by some suspended soil materials, thereby obscuring the color change at the end point. Probably this is due to the increased performance in the modified method of Walkley Black. The indicator should be added just prior to titration to avoid deactivation by adsorption onto clay surfaces. Use of a magnetic stirrer with an incandescent light makes the endpoint easier to see in the turbid system (fluorescent lighting gives a different endpoint color).

Siniakov (1957) proposed that N-phenylanlhranilic acid be used as an indicator in $\text{Cr}_2\text{O}_7^{2-}$ titrations with Fe^{2+} . Mebius (1960) confirmed that N-phenylanlhranilic acid gives a very sharp and clear end point and this compound is currently the indicator of choice for $\text{Cr}_2\text{O}_7^{2-}$ titrations. Our results show that the addition of phosphoric acid before titration (at Turin's method and Tube digestion method) improves the accuracy of determining most standard samples (Table 3).

The behaviour of the discernable change of colour of the indicators to final end point colour was different in different textured soils (Marvat, 1983). In most certified samples the change was clear and sharp with all the three indicators. In sample 7 the change over with ferroin and barium diphenylamine sulfonate was sharp and clear, while with N-phenylanlhranilic acid it was not as clear. In sample 6 the change was clear and sharp only

with barium diphenylamine sulfonate. The probable reason for not getting clear and sharp end point with ferroin may be the turbidity which tends to obscure this colour change at the end point.

Barium diphenylamine sulfonate was the best of all these indicators. N-phenylanthranilic acid can be used with H₃PO₄ addition before titration. Ferroin can be as effective as barium diphenylamine sulfonate if soil is filtered or centrifuged to get clear solution after the addition of water in the procedure, but this process involves a lot of time, hence barium diphenylamine sulfonate and N-phenylanthranilic acid, as indicators, are recommended

4. CONCLUSIONS

SOC content was determined by 10 wet oxidation methods (7 which are commonly used in soil testing laboratories and 3 methods modified by us). The agreement between certified and measured results suggested that SOC could be completely recovered by using wet digestion under tested method described.

Soil organic carbon data obtained from different methods are comparable, except by the Walkley and Black modified method. In spite of small differences in the methodology between the tested titrimetric procedures, used in this study, did not have an important influence on the obtained organic carbon content in the investigated soil samples. Organic carbon contents from the Walkley Black modified method were higher in all investigated samples than those obtained by the other titrimetric methods. The most significant increase is observed in the standards 3, 6 and 7, i.e. the standards with the highest content of organic matter.

The Tube digestion method and Tjurin method (at 1500C and addition of H₃PO₄ before titration) are very effective for soil samples up to 2 % of organic carbon. The Tube digestion method for organic carbon determination seem to be the most suitable methods, for soil samples, compost and sludge with high organic matter content.

Addition of phosphoric acid influences the titrimetric methods. When added during the oxidation, the phosphoric acid promotes the release of calcium from the tightly bonded organic carbon in the composition of humin in more loamy, neutral or slightly alkaline (carbonate) soils, but when added during the titration it blocks the obstructive trivalent iron which is excessively present in the red acidified ferrasols.

The indicators do not significantly affect the quantification of organic carbon in most studied samples. The determined values for all standard samples with indicator diphenylamine are little bit than those determined using the N-phenylanthranilic acid and o-phenanthroline. N-phenylanthranilic acid can be used with H₃PO₄ addition before titration. Ferroin can be as effective as barium diphenylamine sulfonate if soil is filtered or centrifuged to get clear solution after the addition of water in the procedure, but this process involves a lot of time, hence barium diphenylamine sulfonate and N-phenylanthranilic acid, as indicators, are recommended.

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