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## Preface

We would like to present, with great pleasure, the inaugural volume-5, Issue-3, March 2019, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas

### **Environmental Research:**

*Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestrial ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.*

### **Agriculture Research:**

*Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.*

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.



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## Table of Contents

S.No	Title	Page No.
1	<p><b>Application of Citrus Bioadsorbents as Wine Clarifiers</b>  <b>Authors:</b> J. García Raurich, A. Vázquez Ricart, M. Pallarès Andreu, P. Monagas Asensio, M.P. Almajano Pablos</p> <p> <b>DOI:</b> 10.5281/zenodo.2619398</p> <p> <b>Digital Identification Number:</b> IJOEAR-MAR-2019-1</p>	01-11
2	<p><b>Modeling of Soil Organic Carbon Concentration and Stability Variation in Top and Deep Soils with varied Aggregate Size under Climate Change of Sub-tropical India: A Review</b>  <b>Authors:</b> Kancheti Mrunalini, R.K.Naresh, N.C.Mahajan, K.S. Krishna Prasad, Lingutla Sirisha, Sudhir Kumar, S.P.Singh, Shipra Yadav, Jai Ram Chaudhary, Richa Tiwari</p> <p> <b>DOI:</b> 10.5281/zenodo.2619418</p> <p> <b>Digital Identification Number:</b> IJOEAR-MAR-2019-2</p>	12-28
3	<p><b>Genotypic differences of soybean (<i>Glycine max</i> (L.) Merrill) as a factor of biological intensification of agroecosystems</b>  <b>Authors:</b> Sherepitko V.V., Sherepitko D.V.</p> <p> <b>DOI:</b> 10.5281/zenodo.2619424</p> <p> <b>Digital Identification Number:</b> IJOEAR-MAR-2019-3</p>	29-35
4	<p><b>The Cation Exchange Capacity, pH of Soil in Mwogo Marshland, and the Rice Plantation in Huye District –Rwanda</b>  <b>Authors:</b> Innocent Ngiruwonsanga, Abias MANIRAGABA, Fabien MUHIRWA</p> <p> <b>DOI:</b> 10.5281/zenodo.2619430</p> <p> <b>Digital Identification Number:</b> IJOEAR-MAR-2019-4</p>	36-40
5	<p><b>Forage plants in Daloa city livestock market: specific diversity, market practices and economic land</b>  <b>Authors:</b> Amon Anoh Denis-Esdras, Dro Bernadin, Ouattara Pan Issa, Seguena Fofana, Koulibaly Annick Victoire, Grogga Noël, Salla Moreto, Soro Dodiomon</p> <p> <b>DOI:</b> 10.5281/zenodo.2619442</p> <p> <b>Digital Identification Number:</b> IJOEAR-MAR-2019-5</p>	41-47

# Application of Citrus Bioadsorbents as Wine Clarifiers

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**Abstract**— *In recent years, reuse and recycling has taken on an increasingly important role in our society. As a result, there has been an increase in research and development of sustainable technologies. The experience acquired by the CRESCA team in the study of the revaluation of orange peels and lemon have allowed him to have a vision of this by-product as a raw material that, with the opportune treatments, can be origin of products of high added value. In this sense, very satisfactory results have been achieved for different fields of application such as:*

- a) *Agricultural: As water adsorbents, obtaining better results than conventional products (silica gel).*
- b) *Wine: As an alternative wine clarifier to products currently used (gelatin, potato protein, egg albumin, etc.)*
- c) *Treatment of wastewater with high metallic load: As heavy metal adsorbents (Ni, Cu, Pb, etc.)*
- d) *Wastewater Treatment of textile industry: as adsorbent of organic dyes.*

*This paper proposes the use of orange peel and lemon, after being subjected to a process physicochemical, as clarifiers of wine and compared the results with those obtained with vegetable protein, gelatin and bentonite.*

**Keywords**— *absorption, lemon, orange.*

## I. INTRODUCTION

The Spanish law 24/2003 of the Vine and the wine (BOE of 11 July 2003), defines the wine as the natural food obtained exclusively by the alcoholic fermentation, total or partial, of fresh grapes, crushed or not, or of grape must. One of the biggest problems in the manufacture of wine is the residues of grapes, microorganisms and fermentation remains that are deposited in the bottom of the bottle.

Limpidity and stability are achieved both by physical procedures (cold, filtering, centrifugation, racking) and chemical (clarification). While the physical procedures allow to extract or eliminate the particles that cause the turbidity and sedimented microorganisms, thus obtaining the biological stability, the chemists allow to achieve the desired physicochemical stability.

Artificial (or provoked) clarification consists of introducing certain colloidal substances to the wine, which, flocculating, increase their size and deposit themselves in the bottom of the vessels, dragging with them (by adsorption and partly by action Mechanical) the particles scattered in the wine (Hidalgo 2003).

The clarifying agents are selected depending on the item you want to remove. If an excess of astringent and/or drying polyphenolic compounds is detected in the wine, it is advisable to add high molecular weight proteins (such as long-chain gelatines or egg albumin). These will adsorb unwanted compounds and Eliminated by getting a smooth effect on the final wine. On the contrary, if the wine has a protein instability accused it is advisable to add inorganic compounds (such as bentonite or silica gel) so that during the process drag this excess protein and achieve the stability sought (Ribéreau-Gaiusn et al., 1982).

The use of clarifying agents is regulated by Regulation (EC) No 606/2009, which determines the substances that can be used. Currently, they are allowed: food gelatine, protein materials of vegetable origin from wheat, pea and potato; Casein; Fish tail; Potassium caseínatos; Egg albumin; Bentonite; Silicon dioxide in the form of a gel or colloidal solution; Kaolin; Tannins Pectolytic enzymes and enzyme preparations of betaglucanase.

On the other hand, there is a wide variety of materials available in large quantities that have been proposed as adsorbents: natural products, agricultural waste of food industries, among others. In many cases, these residues have been processed to obtain active charcoal, for example, coconut residues (Selomuya et al., 1999) or sugarcane (Mohan and Singh, 2002).



The current trend is the use of agro-industrial waste as an alternative for the preparation of biosorbent materials, since they are cheap and effective in the elimination of heavy metal ions (Fu and Wang, 2011). When processed by physicochemical methods (Vijayaraghavan and Balasubramanian, 2015), cation exchange is the mechanism accepted in the case of the removal of metal ions.

Another area of use of bioadsorption as an alternative process (economic and with acceptable environmental impact) is that of wastewater from the textile industry. Traditionally, these wastewaters have been treated with physical and chemical processes that are costly to eliminate the colorants present. These processes incur operating and maintenance expenses that most small industries are unable to absorb (Lu et al., 2010) (Simphiwe et al., 2012). It should be noted that synthetic dyes are widely used in different types of industries: textiles, paper, pharmaceutical, food, cosmetics, etc., using, approximately 10,000 dyes and pigments of which almost 70% are type azo dyes.

The structural complexity of these xenobiotics compounds translates into a low percentage of elimination of the same in conventional treatment plants, which is why they are discharged without being treated (Gupta and Sahas, 2009). In this way, they provoke different impacts on the environment, producing variations in the waters in terms of suspended solids, ionic load, toxicity, dissolved oxygen concentration, color.

Adsorption is a new treatment option (Wang and Li, 2007) (Afsin, 2007) because it is a substance separation operation, which is done by putting in contact a fluid with a solid adsorbent. This is a surface phenomenon by which the sorbate is retained on the outer surface and the inner pores of the solid (Wang and Zhu, 2007). The superficial retention of these organic molecules is explained by a four-stage mechanism: diffusion of the dye towards the surface of the bioadsorbent; diffusion of the dye through the pore of the bioadsorbent; start of the dye bioadsorption process and final dye bioadsorption process. (Sivakumar and Palanisamy, 2010).

For its part, the chemical procedure of clarification is a process of attraction between the positive loads of the clarifying agents and the negatives of the impurities of the wine so that, by attraction, conglomerates are formed that precipitate at the bottom of the deposits in the form of flocs. This process is carried out after the malolactic fermentation, when the wine presents the highest concentration of solid materials in suspension. The doses used depend on the clarifying agent used and the type of wine treated.

## II. MATERIAL AND METHOD

It was determined the behavior of bioadsorbents obtained by physicochemical treatment of orange peel and lemon and was compared with those of bentonite, vegetable protein and gelatin, products that have been used for many years to reduce proteins present in the wine; astringency due to the presence of tannins, or components that can easily oxidize.

The bentonite was supplied by Agrovín (Alcazar de San Juan, Ciudad Real, Spain), combining a good clarifying action with a high capacity of protein elimination. It is presented as a beige granular.

Laffort España S.L. supplied the vegetable protein used with the name of Vegcoll. It is a clarifier based on vegetable proteins extracted from the potato that is presented in a pulverulent form of beige color. It has a high clarification capacity, a high sedimentation speed and a high elimination of astringent tannins. Gelatine was also supplied by Laffort España S.L. With the name of Gecoll, it is a liquid gelatin of porcine origin. It guarantees a specific action in the elimination of the tannins responsible for the astringency.

The orange and lemon peels were obtained from the local trade. Both were subjected to a physicochemical process through which all sugars contained in the albedo were extracted, as well as the sufficient amount of pectins to achieve a degree of useful consistency to obtain a final product with characteristics of cationic exchanger.

Following the recommendations of the suppliers, the doses of the selected clarifying agents were 18 mg of bentonite; 8mg of gelatin and protein 8 mg in 100 mL of wine. For this reason, the behavior of Bioadsorbents was determined in the following concentrations: 8mg; 13 mg and 18 mg in 100 mL of wine.

### 2.1 Physicochemical treatment of citrus peels

The treatment begins with the collection of orange and lemon peels, followed by a wash with soap and water to remove the wax and resins that are applied to ensure a better appearance for sale.

Once the wash is applied, the shells are dried in an air current at 50 ° C until constant weight, and then the grinding with an ice crusher is obtained, until obtaining particles with a particle size between 500-1000 µm.

With this particle size, chemical treatment is proceeded. It should be noted that lignocellulosic materials are mostly formed by cellulose, hemicellulose, pectin and lignin. These polymers of long, branched or linear chains, are present in the cell walls of plants and are the main responsible for the adsorption of both metal ions (Galant et al., 2014) and macromolecules (Xu et al., 2013).

The chemical treatment begins with a process of acidification of the shells by hydrochloric acid, aimed at the extraction of pectin. Although obtaining pectin from orange peels has been studied extensively (Fishman et al.,2003), (Msebahi et al.,2005), (Liu et al., 2006), (Yeoh et al.,2008), extraction of pectins by conventional methods is carried out at close temperatures at 90 ° C for at least one hour in acidic aqueous solutions (Fishman and Cooke, 2009), so that the pectins are extracted that are not sensitive to calcium. After a while, the resulting solution is removed from the non-soluble solid by filtration. It then mixes with alcohol and precipitates pectin (Claus, 2002).

The extraction of pectin is necessary because it forms colloids and has the property to absorb a large amount of water and, if not, the final product would not have the degree of consistency enough to be used as bioadsorbent. In conjunction with pectin, acid hydrolysis involves solubilization and degradation of carbohydrates, especially xylan and hemicellulose since glucomannan is relatively stable in acidic medium (Van Buren, 1991).

In this study, heat treatment was replaced by ultrasound treatment (US) in order to have a significantly faster methodology (Casas-Orozco et al., 2015) (Sundaraman et al., 2016). For this a US Elmasonic model LC 30 H bath was used with a fixed frequency of 37kHz and regulation of time and temperature, for a period of 45 minutes.

The acid treatment was repeated until obtaining that the remaining solid is free of sugars and soluble pectin, as well as of the fraction of hemicellulose soluble in these experimental conditions. Usually, it is enough with a repetition to get the test of reducing sugars (Fehling method) to be negative. It should be remembered that carbohydrates are components of the cell walls and are part of the structural matrix (Carpita and Mc Cann 2000).

Extracted pectin and hemicellulose soluble in acid medium, as well as reducing sugars, a treatment with distilled water was carried out, in order to remove the excess hydrochloric acid used initially. Next, it was treated in basic medium. In this way, the saponification (demethoxylation) of the non-soluble pectin in acid medium (which still remains in the shell), as well as the solubilization of the soluble fraction of hemicellulose in alkaline medium (Grace et al., 1996), is proceeded. Saponification is usually done with a treatment with NaOH 0.2 M (pH 10-11) under slow agitation for two hours at room temperature. The solid fraction is then filtered and dried is filtered and dried at 50° C.

Once Saponification is produced, the solid fraction is treated with CaCl<sub>2</sub> 0.2M at room temperature, for twenty-four hours keeping it in gentle agitation. This process allows to cross the polymer, to produce the formation of three-dimensional meshes in the internal part in order to increase the mechanical stability of the material.

It is proven (Arjona et al., 2018) that the saponification and cross-linking process can be carried out in a single stage with the use of a dissolution of Ca (OH)<sub>2</sub> 0.2M.

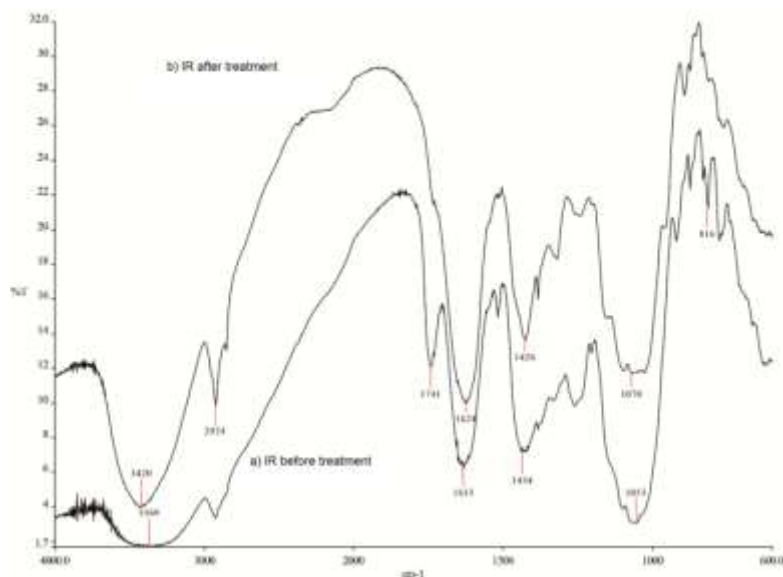
Analogous to what was done in acid treatment, the saponification and crosslinking process performed in this study was carried out in the US bath for a period of 45 minutes. After this treatment, the excess of Ca (II) was eliminated by means of washing with distilled water.

The biopolymers obtained were dried at 105°C and milled until obtaining a final product (bioadsorbent orange based and bioadsorbent lemon based) of particle size between 500-1000 µm.

The verification of the efficacy of this chemical treatment was carried out from the determination of the IR spectrum of the initial shells and the final bioadsorbents.

In Figure 1, the spectra go before and after the chemical treatment is shown, as far as the orange is concerned, made with a spectrophotometer Perkin Elmer model Paragoni 500.

In both spectra, the presence of the functional groups is confirmed: OH, C=O, C-O-C and C-O, as well as C-H and CH<sub>2</sub> before and after chemical treatment, being appreciable the disappearance of a single peak attributable to the extraction of acid-soluble pectin.



**FIGURE 1. a) IR spectrum of Orange peel, b) IR spectrum of the final bioadsorbent**

The broadband signal between ( $3600 - 3200 \text{ cm}^{-1}$ ) centred at  $3369 \text{ cm}^{-1}$  in the case of orange peel before chemical treatment and at  $3420 \text{ cm}^{-1}$  after such treatment is attributable to the hydroxyl group. This group is found in cellulose, pectin, absorbed water, hemicellulose and lignin present in the shell both before and after chemical treatment. The presence of cellulose as the main component of citrus peels makes the elimination of hemicellulose not observable, for practical purposes.

On the other hand, the peak observed at  $2924 \text{ cm}^{-1}$  corresponds to the group C-H and the peaks that appear centered in  $1741 \text{ cm}^{-1}$  and in  $1633 \text{ cm}^{-1}$  that appear in the spectrum of the orange peel, before its chemical treatment are attributable to the carbonyl group ( $\text{C}=\text{O}$ ) as indicators of esterified carboxylic groups and free. In fact, the disappearance of the peak at  $1741 \text{ cm}^{-1}$  in the orange peel spectrum once subjected to chemical treatment indicates the disappearance of the high-methoxyl pectins.

Finally, the peaks centered around  $1430 \text{ cm}^{-1}$  justify the presence of aliphatic and aromatic (C-H) and the peaks centered around  $1060 \text{ cm}^{-1}$  correspond to the C-O group, present in both alcohols and carboxylic acids, and with the ether group (C-O-C).

## 2.2 Spectral characteristics of red wines

Red wines have a maximum absorption at  $520 \text{ nm}$ , where the colour is intense, due to anthocyanins. Between  $280$  and  $520 \text{ nm}$  (2 maximums) there is a minimum (around  $420 \text{ nm}$ ), yellow color zone. As the wine ages, the differences between the two values are diminishing, because the red color disappears and the yellowish shades appear.

The rapid method recommended by the Office International de la Vigne et du Vin (OIV, 2018) determines that the colour intensity (IC) of a wine is the sum of the absorbances of the wine, in a  $1 \text{ cm}$  thick cuvette, corresponding to the wavelengths of  $420 \text{ nm}$ ,  $520 \text{ nm}$  and  $620 \text{ nm}$ . For this reason these wavelengths were chosen to carry out the study. Complementarily, the content of polyphenols was determined with absorbance at  $220 \text{ nm}$ .

The determination of the absorbance was carried out with a molecular absorptive spectrophotometer Agilent model Cary 60 and the experimental results of both bioadsorbents were compared with those obtained with the clarifiers previously selected (bentonite, vegetable protein and gelatin).

## III. RESULTS

### 3.1 Characteristics of cationic exchangers of bioadsorbents

First, we proceeded to check the efficacy of these bioadsorbents as cationic exchangers. To this end, it was verified the elimination of Cu (II) in synthetic dissolutions of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  of different concentrations ( $5, 10, 30, 100 \text{ ppm}$  of  $\text{Cu}^{2+}$ ). To this end,  $25 \text{ mL}$  of each of the four dissolutions of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  were introduced in two test tubes (provided with a screw cap) in which, previously,  $0.5 \text{ g}$  of the corresponding bioadsorbent was added and kept in gentle agitation during an hour.

The determination of Cu (II) was performed with an Atomic absorption spectrometer Varian AA24OFS. The results shown in table 1 make it possible to affirm that both bioadsorbents have a high elimination capacity of Cu (II) and, consequently, present characteristics of cationic exchangers.

**TABLE 1**  
**%BIOADSORPTION OF CU (II) DEPENDING ON THE INITIAL CU (II) CONCENTRATION.**

Bioadsorbent	[Cu(II)] initial mgL <sup>-1</sup>	[Cu(II)] final mgL <sup>-1</sup>	Cu (II) retained (%)
Orange	5	1,25	75
	10	1,15	88,5
	30	2,69	91
	100	3,64	96,4
Lemon	5	0,78	84,4
	10	0,82	91,8
	30	1,64	94,5
	100	2,22	97,8

### 3.2 Clarifying properties of Bioadsorbents

Proven to be effective as cationic exchangers of both bioadsorbents, it was determined its ability to remove organic molecules with electrical load through mechanisms of electrostatic attraction (hydrogen bridges, forces of Van Der Waals...). To this end, we acted on two red wines, one bottled and marketed and another still without bottling.

Experimentally, the centrifugation of a sample of 1L of each type of wine selected by means of a centrifuge Sorvall RC 3b Plus was proceeded. In this way, the suspended solids were eliminated, with a significantly higher sediment in the case of unbottled wine.

The liquid fraction, free of suspended solids, was stored in hermetically sealed glass jars inside a refrigerator at a temperature of 7 °C until the time of use.

The behavior was determined as clarifiers of the bioadsorbent depending on the particle size. This was done by a set of tests with different concentrations and particle sizes of bioadsorbents. The concentrations were: 8mg; 13mg and 18mg in 100mL of wine and particle size: less than 250 µm; between 250-500 µm and higher than 500 µm. Systematically, all the samples were filtered by a Millex filter of 0.45 µm to avoid the possible interference of solids in suspension, proving that the best results were obtained with the particle size between 250-500 µm. Table 2 collects the values of the reduction of absorbance obtained with bottled wine and wine without bottling for the particle size between 250-500 µm.

**TABLE 2**  
**PERCENTAGES OF REDUCTION OF ABSORBANCE OBTAINED WITH BIOADSORBENTS WITH PARTICLE SIZE BETWEEN 250-500 µm (AVERAGE VALUES OF THREE DETERMINATIONS)**

Type of wine	Bioadsorbent	Wavelengths			
		220 nm	420 nm	520 nm	620 nm
Bottled	[Orange]	<b>220 nm</b>	<b>420 nm</b>	<b>520 nm</b>	<b>620 nm</b>
	8 mg/100 mL	10,89 %	1,75 %	2,26%	3,39%
	13 mg/100 mL	18,61 %	2,51 %	3,21 %	4,19 %
	18 mg/100 mL	19,80 %	3,82 %	4,44 %	6,34 %
	[Lemon]	<b>220 nm</b>	<b>420 nm</b>	<b>520 nm</b>	<b>620 nm</b>
	8 mg/100 mL	12,00 %	5,31 %	3,99 %	2,31 %
	13 mg/100 mL	20,29 %	5,54 %	4,25 %	3,51 %
	18 mg/100 mL	21,74 %	7,57 %	5,86 %	5,13 %
Non Bottled	[Orange]	<b>220 nm</b>	<b>420 nm</b>	<b>520 nm</b>	<b>620 nm</b>
	8 mg/100 mL	4,94 %	3,91 %	5,16 %	5,09 %
	13 mg/100 mL	5,38 %	5,91 %	7,37 %	7,75 %
	18 mg/100 mL	6,78 %	7,00 %	8,27 %	9,36 %
	[Lemon]	<b>220 nm</b>	<b>420 nm</b>	<b>520 nm</b>	<b>620 nm</b>
	8 mg/100 mL	6,40 %	5,28 %	6,41 %	6,43 %
	13 mg/100 mL	6,87 %	8,07 %	9,78 %	7,06 %
	18 mg/100 mL	7,54 %	12,48 %	10,67 %	7,77 %

From the results collected in this table it comes off:

- In all cases, the percentage of absorbance reduction is proportional to the concentration of bioadsorbent, both from orange and from lemon.
- In all cases, the percentage of absorbance reduction is higher when the bioadsorbent is the product obtained from lemon.
- In the area of the visible, this percentage is more pronounced in the unbottled wine.
- In the UV zone of the spectrum, this percentage is significantly more pronounced in bottled wine.

### **3.3 Comparison of the behavior of bioadsorbents with respect to Clarifiers**

Once the clarifying capacity of both bioadsorbents was verified, they were compared with vegetable protein, gelatin and bentonite. Selected the Clarifiers and their dosage, the following protocol was followed:

- Preservation of the clarifiers in hermetically sealed containers, under the conditions described by the supplier.
- Preparation of solutions or suspensions of clarifiers.
- Incorporation of the clarifying agent in a progressive way in the wine sample.
- Action of the clarifying agent for long enough, avoiding exceeding 10 days. (During this period of time, the wine remained at absolute rest and at a constant temperature of 7°C inside a refrigerator).
- Careful separation of the sediment from the liquid, just before determining the absorbance, followed by a filtration of the liquid fraction with a Millex filter of 0.45 µm.
- Spectrophotometric determination of the absorbance decrease in the four pre-selected wavelengths.

#### **3.3.1 Behavior of biosorbents with respect to vegetable protein Vgecoll**

First, the behavior of biosorbents compared to the protein Vgecoll was compared, maintaining a constant concentration of the three products studied in 8mg/100 mL of wine.

In the case of non-bottled wine, systematically, the percentage of reduction of the absorbance obtained with the bioadsorbent from the orange peel was lower than that obtained with the bioadsorbent from the lemon peel and with Vgecoll. This behavior was also observed with bottled wine, with the exception of the wavelength of 620 nm in which the three clarifiers presented a behavior without significant differences.

The concentration of the bioadsorbents was then increased to 18 mg/100 mL of wine (maximum recommended concentration for bentonite). In these conditions, the percentage of reduction of the absorbance of bioadsorbents was higher than that of Vgecoll (8mg/100mL of wine), except in the UV zone of the spectrum in the case of unbottled wine.

#### **3.3.2 Behavior of biosorbents with respect to Gecoll gelatin**

In an analogous way to the plant protein, the concentration of the three products studied in 8mg/100 mL of wine was maintained in the first place. In these conditions, in all cases, the percentage of reduction of the absorbance obtained with Gecoll was higher than those obtained with the bioadsorbents.

When the concentration of the bioadsorbents was increased to 18 mg/100 mL of wine, the predominance of the gelatine Gecoll remained, although in a little significant way in the case of bottled wine. On the contrary, in the case of non-bottled wine, bioadsorbents showed values of percentage of absorbance reduction slightly higher than 420 and 620 nm.

#### **3.3.3 Behavior of biosorbents with respect to Bentonite**

On this occasion, the concentration of the three studied products was fixed directly at 18 mg/100 mL.

Bentonite presented the highest percentage of absorbance reduction in the UV zone, surpassing significantly the vegetal protein, gelatin and bioadsorbents, both in bottled wine and non-bottling. As far as the area of the visible spectrum is concerned, only in the case of unbottled wine, both bioadsorbents surpassed the bentonite in the wavelength of 620 nm and the bioadsorbent of lemon at 520 nm.

Table 3 shows the set of experiences with bottled wine and table 4 shows the group of experiences made with unbottled wine.

**TABLE 3**  
**PERCENTAGES OF REDUCTION OF ABSORBANCE OBTAINED IN BOTTLING WINE**

$\lambda$	concentration	Bioadsorbent			Vgcoll	Gecoll	Bentonite
		Orange	Lemon				
220 nm	8mg/100mL	10,89%	12,00%		13,48%	25,00 %	
	13mg/100mL	18,61 %	20,29 %				
	18mg/100mL	19,80 %	21,74 %				<u>38,92%</u>
420 nm	8mg/100mL	1,75 %	2,31 %		2,86 %	<u>6,84%</u>	
	13mg/100mL	2,51%	3,51%				
	18mg/100mL	3,82 %	5,13 %				5,86%
520 nm	8mg/100mL	2,26 %	2,99 %		3,94 %	6,66 %	
	13mg/100mL	3,21%	4,25%				
	18mg/100mL	4,44%	5,86%				<u>8,48 %</u>
620 nm	8mg/100mL	3,39 %	3,42 %		3,68 %	<u>12,96 %</u>	
	13mg/100mL	4,19%	5,54%				
	18mg/100mL	6,34%	7,57%				8,22%

**TABLE 4**  
**PERCENTAGES OF REDUCTION OF ABSORBANCE OBTAINED IN NON-BOTTLE WINE**

$\lambda$	concentration	Bioadsorbent			Vgcoll	Gecoll	Bentonite
		orange	lemon				
220 nm	8mg/100mL	4,95 %	6,40 %		9,84%	9,52 %	
	13mg/100mL	5,38 %	6,87 %)				
	18mg/100mL	6,78 %	7,54 %				<u>27,63%</u>
420 nm	8mg/100mL	3,91 %	5,28 %		6,98 %	6,39 %	
	13mg/100mL	5,91 %	7,06 %				
	18mg/100mL	7,00 %	7,77 %				<u>11,56 %</u>
520 nm	8mg/100mL	5,16 %	6,41 %		7,37 %	<u>11,38 %</u>	
	13mg/100mL	7,47 %	9,78 %				
	18mg/100mL	8,27 %	10,67 %				7,21 %
620 nm	8mg/100mL	5,09 %	6,43 %		6,37 %	8,58 %	
	13mg/100mL	7,75 %	8,07 %				
	18mg/100mL	9,36 %	<u>12,48 %</u>				7,21 %

#### IV. DISCUSSION

The biosorption is a physicochemical process that includes the phenomena of adsorption and absorption of molecules and ions. The biosorption occurs if the molecules or cations of the metals are joined by electrostatic interactions to the anionic sites found in the biosorbents. These sites that serve as active centers for the biosorption are located in the functional groups: carboxyl, hydroxyl, amino, sulfonic, which are part of the structure of most of the polymers of natural origin by different mechanisms ( physical adsorption, complexation, ionic exchange, etc.) (Veglió and Beolchini, 1997; Volesky, 2001; Davis et al., 2003; Gravilescu, 2004; Baytak and Turkes, 2005; Zhang and Banks, 2006).

Biosorbents are natural materials available in large quantities, or certain residual products from industrial or agricultural operations, which can be used for the purpose of capturing contaminants due to their low cost (Vargas et al., 2010).

It has studied a wide range of low cost materials and potential to be used in the biosorption: Wood, clay, ashes, activated sludge, orange peel and banana (Namasivayam et al., 1996).

The lignocellulosic materials (agricultural, agro-industrial and forestry wastes) can present in their composition up to 50% m/m of cellulose, and for that reason have been quite used to obtain that biopolymer and its derivatives, for the production of papers or compounds of high commercial value, such as glucose, ethanol and others (Saha, 2003).

Some of the solids that have been employed are: barks and leaves of conifers, rice husks, walnut, peanut, orange peel, banana peel (Annadurai et al., 2002), Grapefruit husks (Hameed et al., 2008), algae, fungi, nopal, olive bones, etc. (Gupta and Suhas, 2009). However, adsorbents prepared from citrus peels are those that present higher adsorption capacities (Lu et al., 2009).

On the other hand, the clarifying agents must be free from undesirable contaminants and must comply with the applicable legislation. They must be kept in a cool and dry environment, in sealed containers or in other recommended conditions of preservation, according to the manufacturers own suggestion. The quantity of the clarifier used shall be that which corresponds to the lowest level necessary in order to achieve the desired result and in no case must exceed the applicable legislation and standards in force.

The addition of a clarifying agent to wine usually responds to three objectives (resolution OIV, 2014):

- Soften or reduce its astringency and/or bitterness;
- Clarify and eliminate proteins that produce turbidity
- Stabilize and reduce color by adsorption and precipitation of polymerized phenolic compounds and tannins.

The turbid aspect of a wine is due to the presence of scattered particles in it that intercept the luminous radiation that comes from one direction and reflects it in different directions, making them look opaque and turbid. This group of particles that cause turbidity are substances that can be found in the wine or formed during the vinification process (colouring material in colloidal state, potassium tartrate crystals, precipitates of phenolic compounds, proteins...).

Although in the time the decantation can occur in a natural way, in the first months, finished the elaboration, the sedimentation is difficult due to the amount of CO<sub>2</sub> that contains the wine.

The proteins of the wine, contribute to the sensation of unctuousity in the wine, to the stabilization of the foam in sparkling wines (Cava), and they fix the aromas. However, they also provoke the so-called protein bankruptcies. Unstable wine proteins are those of low molecular weight between 12.6-30 kDa and low isoelectric point between 4.1-5.8 (Waters et al., 1992). The process of protein turbidity is caused by denaturation, binding and subsequent flocculation of proteins with other wine compounds in suspension, eventually leading to precipitation in bottled wines (Waters et al., 2005).

As a result of the problems caused by mad cows, there was a movement aimed at substituting the gelatin of animal origin with vegetable proteins.

The protids or proteins are nitrogen substances of complex constitution that in the water give colloidal dispersions. With the pH values of the wine, between 2.8-4 units, they act as electropositive colloids. Employees as clarifiers, are added to the wine in a state of colloidal dispersion, and when coagulating and sedimentation occurs limpidity (Ribéreau-Gaiusn et al., 1984).

The reactions of tannins and protein correspond to flocculation, i.e. the association of particles between them and the formation of flocs which are assembled and precipitated. Proteins that do not react with tannins can be combined with suspended particles or colloidal solution that are negatively charged (most turbid particles in wines have negative charges), producing the Mutual flocculation of the two colloids (neutralization of loads).

In the case of white wines the only really effective system to avoid protein haze is the elimination of unstable proteins, which can only be achieved with the treatment with bentonite or ultrafiltration (Ribéreau-Gaiusn et al., 1999a) ( Hsu ET AL., 1987). However, the clarification with bentonite is a process that affects the sensory quality of the wine. By eliminating most of the proteins, the wine loses structure and unctuousity (Ledoux and Dubourdiou, 1994). In addition, bentonite seriously affects the aroma of wine, since it absorbs directly or indirectly aromas (Guillou et al., 1998), taking into account that proteins are fixatives of aromas and when they are eliminated from the wine, they drag with them some of the aromas (Lubbers et al., 1993) (Lubbers et al., 1996). In addition, proteins are surface molecules and have been found to be very positive factors for foamability and the persistence of foam of sparkling wines (Pueyo et al., 1995) (Brissonet and Maujean, 1993). The alternative of ultrafiltration also significantly affects the aroma and unctuousity of wine (Ribéreau-Gaiusnet.al., 1999b) (Flores et.al., 1991).

The bioadsorbents proposed in this study are constituted by a cellulosic base containing the fraction of pectin not soluble in acid medium. Cellulose is a hygroscopic material, insoluble in water due to its high molecular weight, but it can swell in that medium, being also insoluble in dilute acids, as well as in most organic solvents (Nishinari, 2006). The subsequent alkaline treatment leads to the possibility of a physisorption mechanism because pectin and cellulose have high bonding capacities with the calcium cation.

## V. CONCLUSION

The bioadsorbents obtained from the orange and lemon peels have good clarification capacities, especially the bioadsorbent of lemon.

It cannot be said that there is a universal clarifier, valid for the different components that are determined in the different wavelengths selected

As, chemically, a wine is a complex matrix, the behavior of each clarifier must be considered depending on the type of wine to be clarified.

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# Modeling of Soil Organic Carbon Concentration and Stability Variation in Top and Deep Soils with varied Aggregate Size under Climate Change of Sub-tropical India: A Review

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**Abstract**— The effects of tillage on soil organic carbon (SOC) and nutrient content of soil aggregates can vary spatially and temporally, and for different soil types and cropping systems. Surface soil (0–15 cm) was fractionated into aggregate sizes (>4.76 mm, 4.76–2.00 mm, 2.00–1.00 mm, 1.00–0.25 mm, 0.25–0.053 mm, <0.053 mm) under two tillage regimes. The percentage of soil OC mineralized ( $SOC_{min}$ , % SOC) was in general higher in larger aggregates than in smaller aggregates. Tillage significantly reduced the proportion of macro-aggregate fractions (>2.00 mm) and thus aggregate stability was reduced by 35% compared with RNT, indicating that tillage practices led to soil structural change for this subtropical soil. Soil organic C decreased with increasing soil depth but was greater under tree than others and was mainly concentrated in the topsoil layer (0–20 cm). In comparison to topsoil, deep soil aggregates generally exhibited a lower  $C_{min}$  and higher  $SOC_{min}$ . The highest SOC was in the 1.00–0.25 mm fraction, while the lowest SOC was in micro-aggregate (<0.025 mm) and silt + clay (<0.053 mm) fractions and CT, respectively. Tillage did not influence the patterns in SOC across aggregates but did change the aggregate-size distribution, indicating that tillage affected soil fertility primarily by changing soil structure. The percentage of soil OC mineralized ( $SOC_{min}$ , % SOC) was in general higher in larger aggregates than in smaller aggregates. Meanwhile,  $SOC_{min}$  was greater in coniferous forests (CF) than in broad-leaved forests (BF) at topsoil and deep soil aggregates. In comparison to topsoil, deep soil aggregates generally exhibited a lower  $C_{min}$  and higher  $SOC_{min}$ . The sum of macro-aggregate contributing rates for clay-humus stability of soil organic C (SOC) was significantly superior to that of the micro-aggregates. Water-stable aggregates increased by 34.5% in the CA with residue retention treatment, effectively improving the soil structure. Furthermore, 0.25–1.00 and 1–2mm aggregates had the highest SOC microbial biomass storage and responded rapidly to the various tillage treatments. Greater proportion of micro-aggregates within macro-aggregates in the plots under NT–NT compared with CT–CT was also observed in the surface layer only. Plots under NT–NT had about 10% higher coarse (250–2000  $\mu$ m) intra-aggregate particulate organic matter-C (iPOM–C) within >2000  $\mu$ m sand free aggregates in the 0- to 5-cm soil layer compared with CT–CT plots. The fine (53–250  $\mu$ m) iPOM–C within the 250- to 2000- $\mu$ m aggregates was also higher in the continuous NT plots compared with CT within both >2000 and 250 to 2000  $\mu$ m sand free aggregate size classes in that soil layer.

**Keywords**— Aggregates sizes, aggregate stability, soil depth, macro-aggregates, micro-aggregates, fractionation, particulate organic carbon.

## I. INTRODUCTION

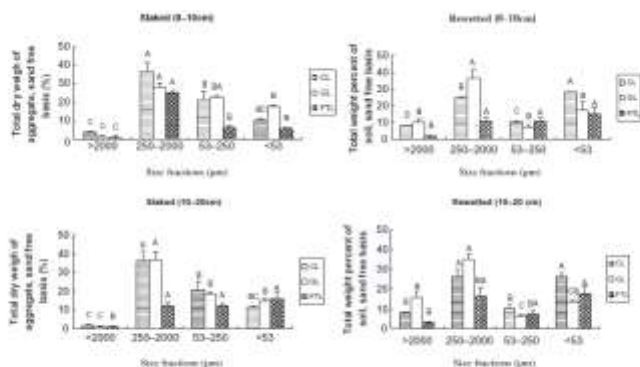
Soil organic carbon (SOC) is the largest constituent of the Earth's terrestrial carbon pool (Stockmann et al., 2013), and slight C losses from the soil may lead to considerable changes in atmospheric CO<sub>2</sub> concentration (Wang et al., 2002), which would affect the magnitude of future climate change (Davidson, and Janssens, 2006). Increasing anthropogenic disturbances especially, on land use/cover change is the major cause of soil quality deterioration in the world (Haynes, 2005). Soil organic carbon (SOC) has recently gained prominence in assessment of soil quality since it compound affects chemical, physical and biological aspects of the soil. Though described by some as the least most understood component of the soil because of its

dynamism, (Lehmann and Kleber, 2015) SOC has been linked to its potential role in carbon sequestration through proper management of land use and cover types (Yang et al., 2012). Land use and cover types influence C fluxes in an ecosystem; through litter quality, deposition and turnover rate. Although SOC is an indicator of soil quality, conceptualization of soil fractions can be used to detect even slight changes in management and regulate degradation (Blair et al., 1995).

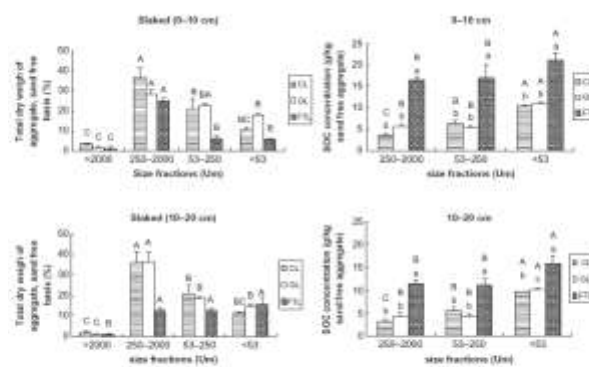
As CO<sub>2</sub> exchange between soil carbon and atmospheric CO<sub>2</sub> varies strongly along climate gradients (Wang et al., 2010) focus on whether there are enhanced response patterns in SOC stability along increasing latitudinal or altitudinal gradients. Numerous studies have implicated temperature as a primary controller of SOC stability by altering the quality and quantity of litter input into soil and soil physico-chemical characteristics (Bird et al., 2002; Garten et al., 2006). SOC stability was found to increase with increasing mean annual temperature (MAT) based on chemical sequential fractionation analysis Hilli et al., 2008). However, the components and stability of SOC were not always consistently related to variations on MAT (Djukic et al., 2010). Radiocarbon dating and <sup>13</sup>C enrichment differentiation for soils indicated that SOC stability along latitudinal and altitudinal gradients was negatively related to MAT (Garten, 2011). Therefore, in addition to temperature affecting SOC stability, other factors must also contribute to SOC stability.

## II. MATERIAL AND METHODS

Dameni et al. (2010) revealed that the distribution of aggregates at the top soil layers (0–10 and 10–20 cm) among the different size classes was significantly influenced by land-use type. Small macro-aggregates (250–2000 μm) represented 15 to 38% and were found to be the dominant aggregates in all land-use types and all soil depths [Fig.1a]. Other size fractions constituted a smaller amount of soil weight, varying from 7% to 22% across land use and soil depths. However, the slaked treatment, large macro-aggregates (>2000 μm) had the smallest amount of soil and represented 1 to 3.5% of the total aggregates collected. In other size classes, the percentages of aggregates collected from different land uses were significantly different and followed the order of <53 μm ≈ 53–250 μm < 250–2000 μm. The distribution of micro-aggregates (53–250 μm) and the mineral fraction (<53 μm) represented 7 to 19% of the total weight of aggregates [Fig.1a]. For the rewetted treatment, the amount of large macro-aggregates (>2000 μm) was consistently greater than the amount collected from the slaked pre-treatment. The percentage of soil collected followed the order of >2000 μm ≈ 53–250 μm < (<53 μm) < 250–2000 μm.



**FIG. 1(a):** Aggregate size distribution for slaked and rewetted pre-treatments, at the top soil layers (0–10 and 10–20 cm) of cropland (CL), forage field (GL), and fruit tree land (FTL) [Source: Dameni et al., 2010].

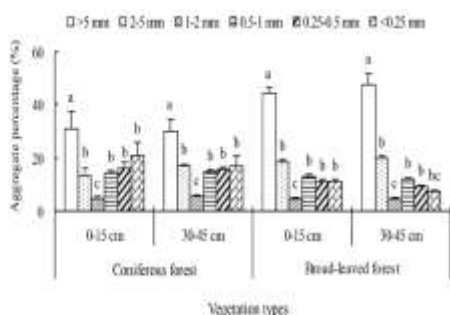


**FIG. 1(b):** Sand-corrected slaked aggregate organic C concentration at the top soil layers (0–10 and 10–20 cm) of cropland (CL), forage field (GL), and fruit tree land (FTL) [Source: Dameni et al., 2010].

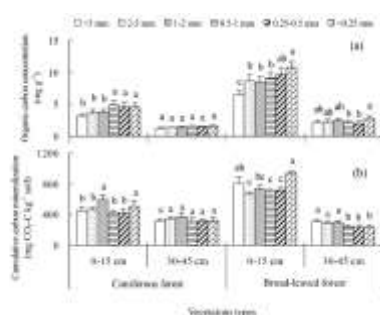
Dameni et al. (2010) also found that the distribution of the SOC contents in aggregate-size fractions at depths of 0–10 and 10–20 cm within the profile under various land-use types [Fig.1b] indicated a significant effect of land use and soil depth on the SOC stock. Within each size class, aggregate C concentration in the FTL soil was significantly greater than in the CL and GL. For each system, differences generally narrowed with increasing soil depth. For FTL soil, a marked decrease in SOC stock from the first to the second layer was noted. In general, the measured SOC stock across land use types was greater in the topsoil (0–10 cm) than in lower depths. Multiple comparisons of means revealed that at the depth of 0–10 cm, the concentration of SOC in different land-use types followed the order FTL > GL ≈ CL for all aggregate-size fractions [Fig.1b].

Fang et al. (2015) also found that the mass of soil aggregates of >5 mm diameter was the greatest followed by 2–5 mm, 0.5–1 mm, 0.25–0.5 mm, and <0.25 mm, and that of 1–2 mm aggregates was the lowest [Fig.2a]. Moreover, smaller aggregates had a higher OC concentration (0.5–1 mm, 0.25–0.5 mm and <0.25 mm) than larger aggregates (>5 mm, 2–5 mm and 1–2 mm) in CF topsoil, and OC concentration decreased with increasing aggregate size in BF topsoil. In contrast, the OC concentration varied very little between aggregate size classes at deep soils in both forests [Fig 2b]. The  $C_{min}$  during the first 15 days was the highest in aggregates of 1–2 mm and <0.25 mm, followed by >5 mm and 2–5 mm, and the lowest in aggregates of 0.5–1 mm and 0.25–0.5 mm in CF topsoil [Fig.2b]. Similarly in BF topsoil, the  $C_{min}$  during the first 15 days was higher in <0.25 mm aggregates than in other aggregates, and did not differ significantly between the six aggregate categories at deeper soil depths in either vegetation type [Fig.2b].

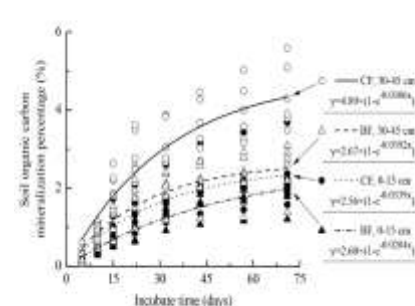
In CF topsoil, the  $C_{min}$  measured over 43 and 71 days were generally higher in aggregates of 1–2 mm and <0.25 mm than in other aggregates, but such patterns were not observed in deep soil. In BF topsoil, the  $C_{min}$  measured over 43 and 71 days were generally higher in aggregates of >5 mm and <0.25 mm than in other aggregates, and higher in larger aggregates (>5 mm, 2–5 mm and 1–2 mm) than in smaller aggregates (0.5–1 mm, 0.25–0.5 mm and <0.25 mm) in deep soils [Fig. 2b].



**FIG. 2(a): The components of soil aggregate fractions of two depths in two restored plantations [Source: Fang et al., 2015]**



**FIG. 2(b): The organic carbon concentration and mineralization of aggregate soil within 71 days at various soil depths in two restored plantations [Source: Fang et al., 2015]**



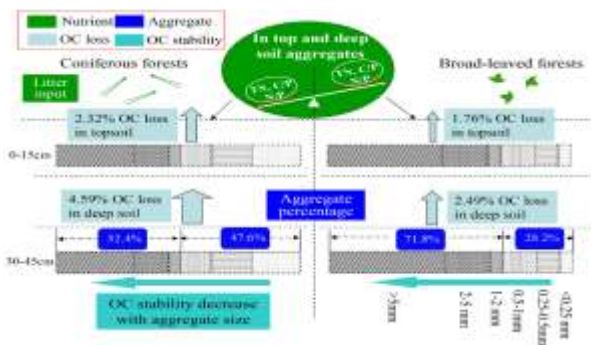
**FIG. 2(c): The weighted mean of soil organic carbon mineralized percentage in various aggregates vary with incubation days in two soil depths under two restored plantations [Source: Fang et al., 2015]**

Fang et al. (2015) revealed that in CF topsoil, the  $SOC_{min}$  was significantly higher in aggregates of 1–2 mm than that in aggregates of 0.5–1 mm and 0.25–0.5 mm, while the highest value of OC mineralization percentage was found in aggregates of >5mm in BF topsoil. Likewise, the soil OC mineralized potential ( $C_0$ ), mineralization constant ( $k$ ) and decomposition days of half mineralizable carbon ( $t_{0.5}$ ) varied with aggregate size, vegetation type and soil depth. The  $C_0$  was higher in CF than in BF soil aggregates at both depths, while the  $t_{0.5}$  in BF topsoil aggregates exceeded those in topsoil aggregates of CF. In CF, the

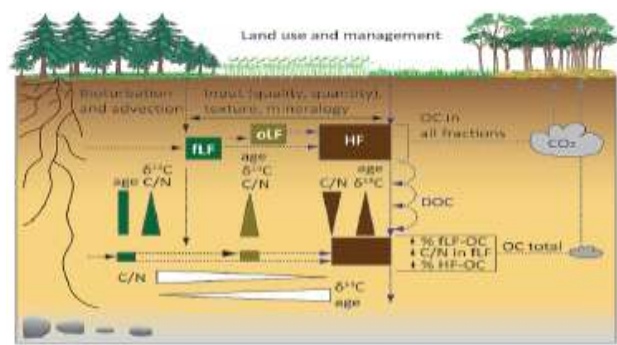
$C_0$  and  $t_{0.5}$  were higher in deep soil aggregates than in topsoil aggregates, however, the  $t_{0.5}$  was lower in deep soil aggregates than in topsoil aggregates in BF [Fig.2c].

Generally, physical protection is one of the important mechanisms to carbon stability. Compared with BF, CF had smaller soil aggregates and fewer larger soil aggregates, and the MWD was lower in CF than that in BF deep soils, which means the stability of the soil OC was better in CF Martens, (2000). However, the value of  $SOC_{min}$  was significantly higher in CF than in BF and there was no difference of  $C_{min}$  in deep soil of CF and BF [Fig. 2b]. Soil organic matters were the adhesive in the formation of soil aggregates [6], which mainly came from root exudates and decomposition of microbes on plant residue Rumpel and Koegel-Knabner, (2011). Soil aggregates might not be a major factor controlling OC stability when soil OC concentration was both low both in CF and BF at the early stages of vegetation restoration. Thus  $SOC_{min}$  was not lower in CF with relatively higher percentage of smaller soil aggregates than in BF.

Blume et al. (2002) reported that microbial activity in deep soil was similar to that measured in topsoil when normalized to biomass size. Therefore, it would not be surprised that deep soil had a higher  $SOC_{min}$  in view of lower OC concentration compared with topsoil [Fig.3a]. Taylor et al. [61] considered that deep soil was metabolically active and contained substantial numbers of microorganisms despite the low biomass contents, which was consistent with the finding that deep soils had a higher value of  $C_{mic}/C_{org}$  quotient (ratio of microbial biomass carbon to OC) Agnelli et al. (2004).



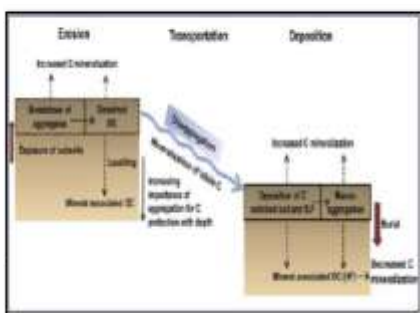
**FIG. 3(a): A stylized illustration of the mechanical framework shows the difference of OC stability influenced by nutrient concentration and aggregate composition in two restored plantations [Source: Fang et al., 2015].**



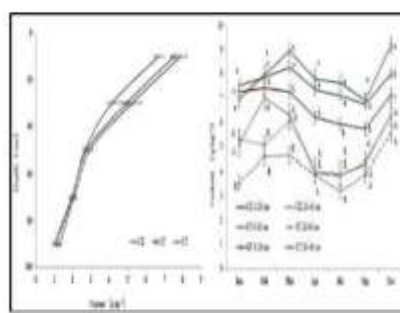
**FIG. 3(b): Characteristics of density fractions in topsoil and subsoil layers and their relation to soil respiration (fLF: free light fraction, oLF: occluded light fraction, HF: heavy fraction, OC: OC concentration) [Source: Schrumpf et al., 2013]**

Schrumpf et al. (2013) also found that the density fractionation separates total soil OC into fractions of different OC-to-TN ratios and HF-OC was in a more advanced decomposition stage than LF-OC, and that more microbial derived OC contributed to HF-OC. This is in line with smaller OC-to-TN-ratios [Fig.3b].

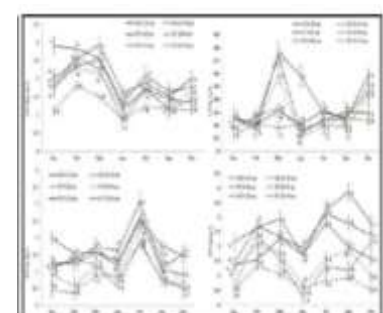
Wang et al. (2014) observed that at both the depositional and the eroding site, the HF represented the most important part of the total SOC at all depths, constituting >80% of SOC [Fig.4a]. The contribution of the HF to SOC was slightly lower at the depositional site than at the eroding site at all depths, indicating the larger contribution of fLF and oLF to SOC at the depositional site. The relative contribution of fLF and oLF to SOC decreased with depth at both sites. No free and occluded light fractions were present at 160-200 cm depth at the eroding site. Gu *et al.* (2016) [revealed that SOC concentration in all treatments decreased with soil depth. The significant differences of SOC among treatments were solely at depths of 0-40 cm, where soil physicochemical properties changed. Further changes would have occurred following activity by microorganisms. Average SOC content at depths of 0-40cm in ST and GT were  $6.26 \text{ g kg}^{-1}$  and  $6.59 \text{ gKg}^{-1}$  respectively, significantly higher than that of  $5.44 \text{ g kg}^{-1}$  in CK [Fig.4b]. The use of ST and GT increased SOC by 15.15% and 21.14% respectively. In the course of the growing season, SOC concentrations in all treatments presented substantial changes with seasons. The maximum SOC was recorded in the dry and cold season, and the minimum in the warm and wet season. Gu *et al.* (2016) also found that compared to the control without cover (CK), ST and GT treatments increased the contents of SOC, LOC, DOC, POC and EOC by 14.73%, 16.5%, 22.5%, 41.5% and 21%, respectively, in the 0-40 cm soil layer, and by 17%, 14%, 19%, and 30%, respectively, in the 0-100 cm soil layer [Fig.4c].



**FIG 4(a): Conceptual model of the interplay between physical and chemical stabilization of soil organic carbon during erosion and deposition [Source: Wang et al., 2014]**



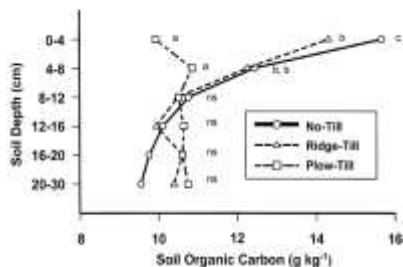
**FIG 4(b): Changes of soil total organic carbon [Source: Gu et al., 2016]**



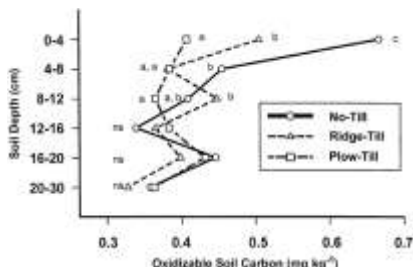
**FIG 4(c): Dynamic changes of carbon fractions [Source: Gu et al., 2016]**

Zibilski et al. (2002) reported that the No-till resulted in significantly greater soil organic C in the top 4 cm of soil, where the organic C concentration was 58% greater than in the top 4 cm of the plow-till treatment. In the 4–8 cm depth, organic C was

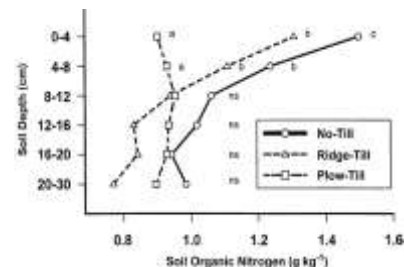
15% greater than the plow-till control [Fig.5a]. The differences were relatively modest, but consistent with organic C gains observed in hot climates where conservation tillage has been adopted. Higher concentrations of total soil N occurred in the same treatments; however a significant reduction in N was detected below 12 cm in the ridge-till treatment [Fig.5b]. The relatively low amount of readily oxidizable C (ROC) in all tillage treatments suggests that much of the soil organic C gained is humic in nature which would be expected to improve C sequestration in this soil [Fig.5c].



**FIG. 5(a): Soil organic carbon by depth after 9 years of no-till, ridge-till or plow-till treatment [Source: Zibilsk et al., 2002]**

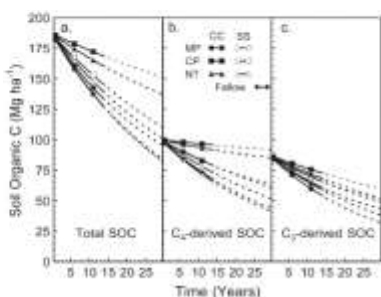


**FIG. 5(b): Readily oxidizable soil carbon by depth after 9 years of no-till, ridge-till or plow-till treatment [Source: Zibilsk et al., 2002]**

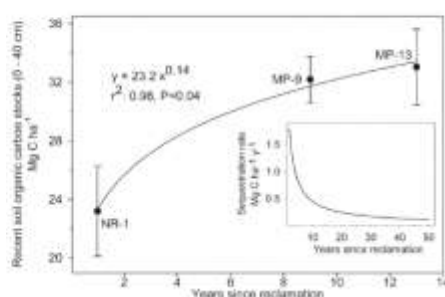


**FIG 5(c): Total soil nitrogen by depth after 9 years of no-till, ridge-till or plow-till treatment [Source: Zibilsk et al., 2002]**

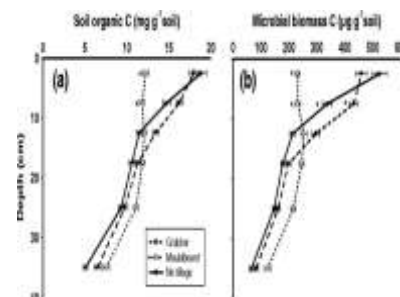
Huggins et al. (2014) revealed that in addition to less C inputs than CC, SS accelerated rates of SOC decomposition. Tillage effects on SOC were greatest in CC where CP had 26% and NT 20% more SOC than MP, whereas SOC in SS was similar across tillage treatments [Fig.6a]. Up to 33% of the greater SOC under CC for CP and NT, compared with MP, occurred below tillage operating depths. Jacinthe and Lal, (2009) concluded that the rates of C sequestration were estimated from the temporal trend in the recent SOC pool (0– 40 cm in NR (23.2 Mg C ha<sup>-1</sup>), 9-yr MP (32.9 Mg C ha<sup>-1</sup>) and 13-yr MP (33 Mg C ha<sup>-1</sup>), and ranged between 0.8 and 0.25 Mg C ha<sup>-1</sup> yr<sup>-1</sup> during the first and second decades of restoration. Despite a similar amount of crop residue returned (2.8 Mg C ha<sup>-1</sup> yr<sup>-1</sup>), recent SOC under 13-yr NT (36.8 Mg C ha<sup>-1</sup>) exceeded that under 13-yr MP by 3.8 Mg C ha<sup>-1</sup> [Fig.6b]. Murugan et al. (2013) revealed that the GRT and NT treatments increased the stocks of SOC (+7 %) and microbial biomass C (+20 %) in comparison with the MBT treatment. The differences between the GRT and NT were small, but there were more positive effects for the GRT treatment in most cases (Fig.6c).



**FIG. 6(a): Thirty year simulation of tillage and crop sequence effects on total soil organic C, C<sub>4</sub> derived SOC and C<sub>3</sub>-derived SOC [Source: Huggins et al., 2014].**



**FIG. 6(b): Temporal evolution of recent organic carbon (SOC) pools in mineland reclaimed to agricultural land-use under conventional tillage [Source: Jacinthe and Lal, 2009]**



**FIG. 6(c): Influence of different tillage intensities on soil microbial biomass at different depths (Source: Murugan et al., 2013)**

Naresh et al. (2017) reported that the T<sub>3</sub> treatment resulted in significantly increased 66.1%, 50.9%, 38.3% and 32% LFOC, PON, LFON and POC, over T<sub>7</sub> treatment and WSC 39.6% in surface soil and 37.4% in subsurface soil [Table 1].LFOC were also significantly higher following the treatments including organic amendment than following applications solely of chemical fertilizers, except that the F<sub>5</sub>, F<sub>6</sub> and F<sub>7</sub> treatments resulted in similar LFOC contents. Application solely of chemical fertilizers had no significant effects on LFOC compared with unfertilized control plots. Nevertheless, application of F<sub>5</sub> or F<sub>6</sub> significantly increased contents of POC relative to F<sub>1</sub> (by 49.6% and 63.4%, respectively). Rajan et al. (2012) concluded that FYM can increase the root biomass and microbial biomass debris which is the main source of POC. It is suggested that the greater biochemical recalcitrance of root litter. Puget et al. (1995) might have also increased the POC contents in soil depending upon the root biomass produced. The continuous replacement of organic manure on the soil

creates a favorable environment for the cycling of C and formation of macro-aggregates. Furthermore, POC acts as a cementing agent to stabilize macro-aggregates and protect intra-aggregate C in the form of POC Six et al., (2002).

**TABLE 1**  
**EFFECT OF 15 YEARS OF APPLICATION OF TREATMENTS ON CONTENTS OF VARIOUS LABILE FRACTIONS OF CARBON IN SOIL [NARESH ET AL., 2017]**

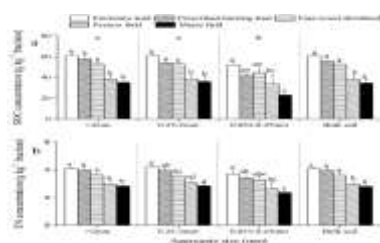
Treatments	0-5 cm layer					5-15 cm layer				
	WSC (mgkg <sup>-1</sup> )	POC (mgkg <sup>-1</sup> )	PON (mgkg <sup>-1</sup> )	LFOC (mgkg <sup>-1</sup> )	LFON (mgkg <sup>-1</sup> )	WSC (mgkg <sup>-1</sup> )	POC (mgkg <sup>-1</sup> )	PON (mgkg <sup>-1</sup> )	LFOC (mgkg <sup>-1</sup> )	LFON (mgkg <sup>-1</sup> )
<b>Tillage crop residue practices</b>										
T <sub>1</sub>	23.9 <sup>ad</sup>	638 <sup>a</sup>	67.2 <sup>a</sup>	81.3 <sup>ad</sup>	9.1 <sup>d</sup>	15.7 <sup>d</sup>	535 <sup>a</sup>	54.7 <sup>a</sup>	65.1 <sup>d</sup>	7.8 <sup>d</sup>
T <sub>2</sub>	25.9 <sup>c</sup>	898 <sup>bc</sup>	88.6 <sup>cd</sup>	107.8 <sup>bc</sup>	11.8 <sup>c</sup>	17.8 <sup>cd</sup>	674 <sup>cd</sup>	74.5 <sup>cd</sup>	94.1 <sup>bc</sup>	9.1 <sup>c</sup>
T <sub>3</sub>	27.8 <sup>ab</sup>	1105 <sup>ab</sup>	106.7 <sup>ab</sup>	155.2 <sup>a</sup>	13.3 <sup>ab</sup>	19.6 <sup>bc</sup>	785 <sup>bc</sup>	91.6 <sup>ab</sup>	132.6 <sup>a</sup>	10.9 <sup>ab</sup>
T <sub>4</sub>	22.7 <sup>d</sup>	779 <sup>cd</sup>	77.9 <sup>d</sup>	95.7 <sup>c</sup>	9.8 <sup>d</sup>	17.6 <sup>cd</sup>	609 <sup>de</sup>	69.1 <sup>de</sup>	87.6 <sup>c</sup>	8.3 <sup>cd</sup>
T <sub>5</sub>	26.4 <sup>bc</sup>	1033 <sup>b</sup>	97.4 <sup>bc</sup>	128.8 <sup>b</sup>	12.6 <sup>bc</sup>	20.3 <sup>ab</sup>	842 <sup>ab</sup>	87.3 <sup>bc</sup>	102.0 <sup>b</sup>	10.4 <sup>b</sup>
T <sub>6</sub>	29.2 <sup>a</sup>	1357 <sup>a</sup>	117.5 <sup>a</sup>	177.8 <sup>a</sup>	14.2 <sup>a</sup>	22.6 <sup>a</sup>	974 <sup>a</sup>	106.1 <sup>a</sup>	141.2 <sup>a</sup>	11.8 <sup>a</sup>
T <sub>7</sub>	17.2 <sup>e</sup>	620 <sup>d</sup>	22.5 <sup>e</sup>	52.7 <sup>e</sup>	8.2 <sup>d</sup>	13.2 <sup>e</sup>	485 <sup>e</sup>	18.8 <sup>f</sup>	49.8 <sup>f</sup>	6.8 <sup>e</sup>
<b>Nutrient Management Practices</b>										
F <sub>1</sub>	21.9 <sup>e</sup>	631 <sup>a</sup>	24.7 <sup>e</sup>	89.2 <sup>c</sup>	6.8 <sup>d</sup>	15.1 <sup>e</sup>	585	17.3 <sup>e</sup>	47.9 <sup>f</sup>	5.9 <sup>e</sup>
F <sub>2</sub>	29.2 <sup>ad</sup>	869 <sup>c</sup>	92.5 <sup>c</sup>	96.4 <sup>c</sup>	9.5 <sup>c</sup>	20.2 <sup>cd</sup>	789	73.5 <sup>cd</sup>	85.9 <sup>d</sup>	8.0 <sup>c</sup>
F <sub>3</sub>	29.8 <sup>c</sup>	956 <sup>bc</sup>	96.8 <sup>c</sup>	108.1 <sup>bc</sup>	10.5 <sup>bc</sup>	21.9 <sup>bc</sup>	813	79.4 <sup>c</sup>	96.9 <sup>cd</sup>	9.6 <sup>bc</sup>
F <sub>4</sub>	28.4 <sup>d</sup>	788 <sup>cd</sup>	72.9 <sup>d</sup>	91.3 <sup>c</sup>	7.9 <sup>d</sup>	18.8 <sup>d</sup>	728	59.4 <sup>d</sup>	66.7 <sup>e</sup>	7.2 <sup>d</sup>
F <sub>5</sub>	32.5 <sup>a</sup>	1381 <sup>a</sup>	130.8 <sup>a</sup>	183.9 <sup>a</sup>	13.8 <sup>a</sup>	26.4 <sup>a</sup>	1032 <sup>a</sup>	112.1 <sup>a</sup>	152.9 <sup>a</sup>	12.4 <sup>a</sup>
F <sub>6</sub>	31.6 <sup>ab</sup>	1156 <sup>ab</sup>	114.2 <sup>ab</sup>	160.5 <sup>a</sup>	12.6 <sup>ab</sup>	23.6 <sup>ab</sup>	905 <sup>ab</sup>	96.7 <sup>ab</sup>	139.7 <sup>a</sup>	11.9 <sup>a</sup>
F <sub>7</sub>	30.9 <sup>b</sup>	1102 <sup>b</sup>	103.9 <sup>bc</sup>	123.4 <sup>b</sup>	11.5 <sup>ab</sup>	22.7 <sup>ab</sup>	826 <sup>b</sup>	88.3 <sup>bc</sup>	103.2 <sup>bc</sup>	10.1 <sup>b</sup>

Values in a column followed by the same letter are not significantly different ( $P < 0.05$ ).

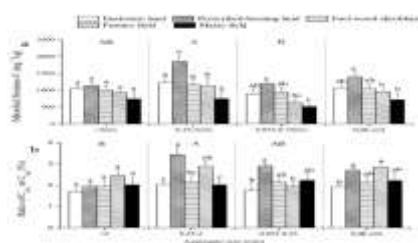
WSC = water soluble C, POC = particulate organic C, PON = particulate organic N, LFOC = light fraction organic C, and LFON = light fraction organic N.

Xiao et al. (2016) showed that the SOC concentrations were significantly higher in macro-aggregates than micro-aggregates; the MBC and  $C_{mic}$ :  $C_{org}$  ratios were highest in small macro-aggregates. Therefore, small macro-aggregates might have more active C dynamics [Fig.7a]. In agricultural ecosystems, decreases in SOC are mainly induced by frequent soil disturbance (e.g. tillage, fertilization, and weed control) and crop removal (Kocyigit and Demirci, 2012). MBC in aggregates and bulk soil in other land uses decreased compared with that in enclosure land [Fig.7b]. Further, the maize field had the lowest MBC. Moreover, the MBC in small micro-aggregates of prescribed-burning land (1850.62 mg kg<sup>-1</sup>) was significantly higher than that of enclosure land (1219.90 mg kg<sup>-1</sup>). The pasture and maize fields had much lower MBC in micro-aggregates (623.36 mgkg<sup>-1</sup> and 514.30 mgkg<sup>-1</sup>, respectively). However, the MBC in large macro-aggregates did not differ significantly among all land uses. In the three aggregates, MBC was the highest in small macro-aggregates, followed by large macro-aggregates and micro-aggregates [Fig.7b]. The  $C_{mic}$ :  $C_{org}$  ratios ranged between 1.71% and 3.44% [Fig.7b]. Compared to enclosure land, the ratios in other land uses increased in aggregates and bulk soil. The highest  $C_{mic}$ :  $C_{org}$  ratio (3.44%) was observed in small macro-aggregates. This is mainly because the large radius of large aggregates could limit the O<sub>2</sub> concentration and gas diffusion required by microbes (Gupta and Germida, 2015; Jiang et al., 2011). Thus, large macro-aggregates might diminish the impacts of land uses and facilitate the maintenance of a stable microbial biomass.

Dou et al. (2008) reported that SMBC was 5 to 8%, mineralized C was 2%, POM C was 14 to 31%, hydrolyzable C was 53 to 71%, and DOC was 1 to 2% of SOC. No-till significantly increased SMBC in the 0- to 30-cm depth, especially in the surface 0 to 5 cm [Fig.7c]. Under NT, SMBC at 0 to 5 cm was 25, 33, and 22% greater for CW, SWS, and WS, respectively, than under CT, but was 20 and 8% lower for CW and WS, respectively, than under CT at the 5- to 15-cm depth. At the 15- to 30-cm depth, no consistent effect of tillage was observed [Fig7c].



**Fig. 7(a):** Soil organic carbon (SOC) (a) and total nitrogen (TN) (b) concentrations in the three sizes of soil aggregates and in bulk soil of different land uses [Source: Xiao et al., 2016]



**Fig.7 (b):** Microbial biomass carbon (MBC) (a) and the  $C_{mic}$ :  $C_{org}$  ratios (b) of the three sizes of soil aggregates and bulk soil of different land uses [Source: Xiao et al., 2016]



**Fig. 7(c):** Soil microbial biomass C (SMBC) and its proportion of soil organic C (SOC) as affected by cropping sequence and tillage at 0- to 5-, 5- to 15-, and 15- to 30-cm depths [Source: Dou et al., 2008]



Kumar et al. (2018) also found that the ZTR (zero till with residue retention) ( $T_1$ ) and RTR (Reduced till with residue retention) ( $T_3$ ) showed significantly higher BC, WSOC, SOC and OC content of 24.5%, 21.9%, 19.37 and 18.34  $g\ kg^{-1}$ , respectively [Table 2] as compared to the other treatments. Irrespective of residue retention, wheat sown in zero till plots enhanced 22.7%, 15.7%, 36.9% and 28.8% of BC, WSOC, SOC and OC, respectively, in surface soil as compared to conventional tillage [Table 2]. Simultaneously, residue retention in zero tillage caused an increment of 22.3%, 14.0%, 24.1% and 19.4% in BC, WSOC, SOC and OC, respectively over the treatments with no residue management. Similar increasing trends of conservation practices on different forms of carbon under sub-surface (15–30 cm) soil were observed however, the magnitude was relatively lower [Table 2]. Zhu *et al.*, (2011) compared to conventional tillage (CT) and zero-tillage (ZT) could significantly improve the SOC content in cropland. Frequent tillage under CT easily exacerbate C-rich macro-aggregates in soils broken down due to the increase of tillage intensity, then forming a large number of small aggregates with relatively low organic carbon content and free organic matter particles. Free organic matter particles have poor stability and are easy to degradation, thereby causing the loss of SOC Song *et al.*, (2011).

**TABLE 2**  
**EFFECT OF TILLAGE AND NITROGEN MANAGEMENT ON DISTRIBUTION OF DIFFERENT FORMS OF CARBON IN SOIL [KUMAR ET AL., 2018]**

Treatments	WSOC ( $g\ kg^{-1}$ )		SOC ( $g\ kg^{-1}$ )		OC ( $g\ kg^{-1}$ )		BC ( $g\ kg^{-1}$ )	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
<b>Tillage Practices</b>								
$T_1$ ZTR	28.8	26.2	23.1	19.3	9.61	9.13	4.69	4.28
$T_2$ ZTWR	25.3	24.6	18.4	14.8	7.87	7.21	3.76	3.19
$T_3$ RTR	27.0	25.9	22.4	18.2	8.68	8.17	4.13	3.87
$T_4$ RTWR	23.7	21.8	18.1	14.2	7.66	7.07	3.12	2.96
$T_5$ CTR	26.1	24.4	21.8	17.4	8.49	7.96	3.82	3.48
$T_6$ CT	21.8	20.9	16.1	13.1	6.21	5.64	2.89	2.63
<b>Nitrogen Management</b>								
$F_0$ Control	21.1	14.9	16.1	13.1	6.13	5.48	1.58	1.07
$F_1$ 80 $kg\ N\ ha^{-1}$	28.3	21.2	17.8	14.7	6.46	6.16	2.46	1.75
$F_2$ 120 $kg\ N\ ha^{-1}$	29.5	22.1	19.1	16.1	7.25	6.71	3.26	2.18
$F_3$ 160 $kg\ N\ ha^{-1}$	30.2	23.1	20.8	18.2	7.75	7.28	3.82	2.66
$F_4$ 200 $kg\ N\ ha^{-1}$	31.1	25.4	21.3	18.7	7.93	7.48	4.15	3.42

Different small letters within the same column show the significant difference at  $P = 0.05$  according to Duncan Multiple Range Test for separation of mean.

WSOC= Water soluble organic carbon, SOC =Total soil organic carbon, OC =Oxidizable organic carbon, BC =Black carbon

Kumar et al. (2018) revealed that at the 0–15 and 15-30 cm, POC, PON, LFOC and LFON content under ZT and RT with residue retention was greater than under without residue and conventional sown plots, respectively. The decrease in the disruption of soil macro-aggregates under ZT plots permitted a greater accumulation of SOC between and within the aggregates. Thus, less soil disturbance is the major cause of higher POC in the ZT and RT plots compared with the CT plots in the 0-15cm and 15-30 cm soil layers [Table 3]. This phenomenon might lead to micro-aggregate formation within macro-aggregates formed around fine intra-aggregate POC and to a long-term stabilization of SOC occluded within these micro-aggregates. The sequestration rate of POC, PON, LFOC and LFON in all the treatments followed the order 200  $kg\ N\ ha^{-1}$  ( $F_4$ ) > 160  $kg\ N\ ha^{-1}$  ( $F_3$ ) > 120  $kg\ N\ ha^{-1}$  ( $F_2$ ) > 80  $kg\ N\ ha^{-1}$  ( $F_1$ ) > control (unfertilized) ( $F_0$ ) [Table 3]. Chen et al., (2009) also found that single effect of residue application was not significant but its significance became apparent after its interaction with tillage system.

**TABLE 3**  
**EFFECT OF DIFFERENT TREATMENTS ON CONTENTS OF VARIOUS LABILE FRACTIONS OF CARBON IN SOIL [KUMAR ET AL., 2018]**

Treatments	POC ( $mg\ kg^{-1}$ )		PON ( $mg\ kg^{-1}$ )		LFOC ( $mg\ kg^{-1}$ )		LFON ( $mg\ kg^{-1}$ )	
	0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm
<b>Tillage Practices</b>								
$T_1$ ZTR	1342.8	967.9	119.5	108.1	194.7	154.8	14.8	12.3
$T_2$ ZTWR	981.1	667.4	94.6	86.5	120.5	104.7	11.8	10.3
$T_3$ RTR	1230.2	836.9	109.7	97.8	170.9	144.9	13.7	11.6
$T_4$ RTWR	869.4	604.4	82.6	76.6	107.1	97.3	9.7	8.6
$T_5$ CTR	1099.1	779.4	98.4	89.3	143.8	115.9	12.8	10.9
$T_6$ CT	617.5	481.8	69.2	57.6	90.8	73.6	9.6	7.9
<b>Nitrogen Management</b>								
$F_0$ Control	709.7	658.6	31.7	26.3	123.9	104.3	6.4	5.8
$F_1$ 80 $kg\ N\ ha^{-1}$	860.7	785.6	68.4	56.2	132.8	116.1	7.6	6.9
$F_2$ 120 $kg\ N\ ha^{-1}$	952.2	808.9	89.5	78.5	150.6	127.6	9.7	8.6
$F_3$ 160 $kg\ N\ ha^{-1}$	1099.5	823.8	96.8	83.4	168.5	145.7	10.2	9.8
$F_4$ 200 $kg\ N\ ha^{-1}$	1153.1	898.4	103.9	97.3	176.2	152.9	11.7	10.6

Values in a column followed by the same letter are not significantly different ( $P < 0.05$ ).

POC = particulate organic carbon, PON = particulate organic nitrogen, LFOC = labile fraction organic carbon, and LFON = labile fraction organic nitrogen.

Zheng et al. (2018) observed that the SOC storage in macro-aggregates under different treatments significantly decreased with soil depth [Table 4]. However, no significant variation was observed in the micro-aggregate associated C storage with depth. SOC storage increased with aggregate size from  $1 \pm 2$  to  $> 2$  mm and decreased with a decrease in aggregate size. The SOC storage in macro-aggregates of all sizes from 0-30cm depth was higher in the ST treatment than in other treatments. From 30-60cm, trends were less clear. SOC storage in micro-aggregates showed the opposite trend, with significantly higher levels in the CT treatment from 0-30cm, and no significant differences between treatments below this depth. Soil aggregates have three major effects on soil (Kladivko, 2001). They regulate and maintain water, fertilizer, gas, and heat in the soil, affect the types and activity of the soil enzymes, and also maintain and stabilize the loose arable layer (Ismail et al., 1994). Almost 90% of SOC exists in the form of aggregates in the topsoil. Protection and maintenance of the macro-aggregate stability and ratio are of great importance in the sustainability of soil fertility (Nimmo and Perkins, 2002). In addition, the contributing rate of SOC in differently sized aggregates decreased, consistent with the trend of soil aggregate-associated C storage and SOC with increasing soil depth.

Naresh et al. (2016) also found significantly higher POC content was probably also due to higher biomass C. Results on PON content after 3-year showed that in 0-5 cm soil layer of CT system,  $T_1$ , and  $T_5$  treatments increased PON content from  $35.8 \text{ mg kg}^{-1}$  in CT ( $T_0$ ) to 47.3 and  $67.7 \text{ mg} \cdot \text{kg}^{-1}$  without CR, and to 78.3, 92.4 and  $103.8 \text{ mg kg}^{-1}$  with CR @ 2, 4 and  $6 \text{ t ha}^{-1}$ , respectively. The corresponding increase of PON content under CA system was from  $35.9 \text{ mg kg}^{-1}$  in CT system to 49 and  $69.6 \text{ mg kg}^{-1}$  without CR and 79.3, 93.0 and  $104.3 \text{ mg kg}^{-1}$  with CR @ 2, 4 and  $6 \text{ t ha}^{-1}$ , respectively. Small improvement in PON content was observed after 4 years of the experiment. Singh et al. (2014) found that carbon stock of 18.75, 19.84 and  $23.83 \text{ Mg ha}^{-1}$  in the surface 0.4 m soil depth observed under CT was increased to 22.32, 26.73 and  $33.07 \text{ Mg ha}^{-1}$  in 15 years of ZT in sandy loam, loam and clay loam soil. This increase was highest in clay loam (38.8%) followed by loam (34.7%) and sandy loam (19.0%) soil. The carbon sequestration rate was found to be 0.24, 0.46 and  $0.62 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  in sandy loam, loam and clay loam soil under ZT over CT. Thus, fine textured soils have more potential for storing carbon and ZT practice enhances carbon sequestration rate in soils by providing better conditions in terms of moisture and temperature for higher biomass production and reduced oxidation (Gonzalez-Sanchez et al., 2012). Gupta Choudhury et al. (2014) revealed that the residue incorporation or retention caused a significant increment of 15.65% in total water stable aggregates in surface soil (0–15 cm) and 7.53% in sub-surface soil (15–30 cm), which depicted that residue management could improve 2.1-fold higher water stable aggregates as compared to the other treatments without residue incorporation/retention. Bhattacharya et al. (2013) reported that tillage-induced changes in POM C were distinguishable only in the 0- to 5-cm soil layer; the differences were insignificant in the 5- to 15-cm soil layer. Plots under ZT had about 14% higher POM C than CT plots ( $3.61 \text{ g kg}^{-1}$  bulk soil) in the surface soil layer.

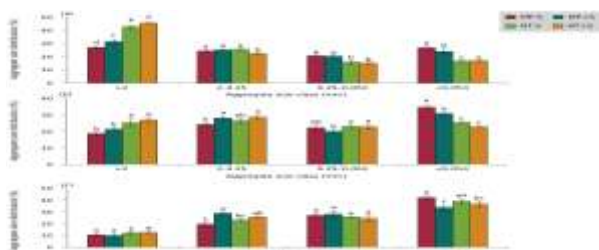
**TABLE 4**  
**DISTRIBUTION OF SOIL ORGANIC CARBON STORAGE IN WATER-STABLE AGGREGATES IN DIFFERENT SOIL LAYERS AND TILLAGE TREATMENTS [ZHENG ET AL., 2018]**

Depth (cm)	Treatments	Macro-aggregate ( $\text{t ha}^{-1}$ )				Micro-aggregate ( $\text{t ha}^{-1}$ )			
		> 2 mm	2-1 mm	1-0.25 mm	Sum	0.25-0.053 mm	0.053-0.002 mm	< 0.002 mm	Sum
0-10	ST	2.65±0.74a*	5.87±0.34a	7.75±0.23a	16.28±0.85a	1.38±0.11c	0.26±0.02c	0.26±0.08b	1.90±0.08c
	NT	1.40±0.07b	5.82±0.36a	7.78±0.40a	15.00±0.11a	1.26±0.10c	0.23±0.02c	0.25±0.04b	1.75±0.08c
	MP	0.35±0.01b	3.98±0.29b	5.91±0.43b	10.24±0.17b	2.44±0.06b	0.73±0.05b	0.69±0.07a	3.86±0.08b
	CT	0.44±0.04b	4.43±0.22b	6.11±0.54b	10.99±0.37b	2.88±0.08a	1.96±0.23a	0.44±0.14ab	5.28±0.20a
10-20	ST	2.43±0.03a	6.85±0.19a	9.14±0.16ab	18.42±0.29a	0.61±0.01ab	1.54±0.10c	0.72±0.01ab	2.86±0.11b
	NT	1.62±0.02b	5.04±0.25b	8.49±0.10b	15.15±0.22b	0.49±0.10b	1.40±0.03c	0.67±0.14b	2.56±0.27b
	MP	0.59±0.03d	4.02±0.31c	7.67±0.31c	12.28±0.16c	0.82±0.01a	3.27±0.06b	0.97±0.02ab	5.05±0.07a
	CT	1.35±0.09c	4.69±0.09bc	9.42±0.19a	15.46±0.36b	0.73±0.11ab	3.56±0.08a	1.05±0.17a	5.35±0.23a
20-30	ST	3.06±0.10a	6.77±0.51a	9.92±0.17a	19.75±0.47a	1.70±0.56a	0.96±0.28b	0.21±0.11c	2.87±0.44b
	NT	1.41±0.03b	6.32±0.47a	8.30±0.10ab	16.02±0.34c	1.99±0.13a	0.98±0.10b	0.54±0.11bc	3.51±0.32b
	MP	2.15±0.26b	6.52±1.23a	9.03±1.10ab	17.71±0.38b	2.03±0.22a	0.59±0.21b	0.59±0.06b	3.20±0.37b
	CT	2.09±0.46b	3.48±0.36b	7.76±0.11b	13.33±0.07d	1.88±0.07a	1.73±0.09a	2.12±0.14a	5.73±0.06a
30-40	ST	1.92±0.03a	5.74±0.61a	7.01±0.57a	14.67±0.09a	1.29±0.26a	0.68±0.24a	0.33±0.04a	2.31±0.10a
	NT	1.06±0.25ab	4.00±0.54a	4.43±0.15b	9.50±0.34b	1.27±0.15a	0.93±0.34a	0.26±0.10a	2.45±0.27a
	MP	1.12±0.45ab	4.71±0.42a	7.72±0.57a	13.56±0.23a	1.20±0.06a	0.56±0.14a	0.31±0.12a	2.07±0.12a
	CT	0.60±0.14b	2.87±1.53a	5.83±1.19ab	9.30±1.01b	2.00±0.58a	0.95±0.26a	0.10±0.02a	3.05±0.86a
40-50	ST	0.66±0.23ab	3.29±0.90a	4.60±0.55a	8.55±0.39a	0.79±0.35a	0.48±0.18a	0.26±0.06a	1.53±0.58a
	NT	0.23±0.07b	1.66±0.24a	4.02±0.36ab	5.90±0.23c	1.09±0.26a	0.16±0.04a	0.21±0.06a	1.46±0.35a
	MP	0.87±0.24a	2.97±0.60a	3.35±0.26b	7.18±0.27b	0.93±0.16a	0.25±0.19a	0.34±0.07a	1.53±0.26a
	CT	0.55±0.19ab	1.71±0.20a	4.85±0.04a	7.11±0.33b	1.35±0.29a	0.33±0.11a	0.15±0.06a	1.83±0.27a
50-60	ST	0.23±0.15a	1.99±0.21a	3.48±0.31a	5.69±0.05a	0.80±0.04b	0.22±0.04b	0.33±0.06a	1.34±0.12b
	NT	0.34±0.07a	1.06±0.06b	3.50±0.17a	4.90±0.06b	1.33±0.08a	0.19±0.04b	0.17±0.03a	1.69±0.10b
	MP	0.31±0.11a	2.21±0.25a	3.20±0.35ab	5.72±0.14a	1.29±0.03a	0.20±0.06b	0.23±0.07a	1.71±0.15b
	CT	0.15±0.03a	1.83±0.10a	2.38±0.06b	4.36±0.05c	1.21±0.02a	0.96±0.06a	0.26±0.04a	2.44±0.12a

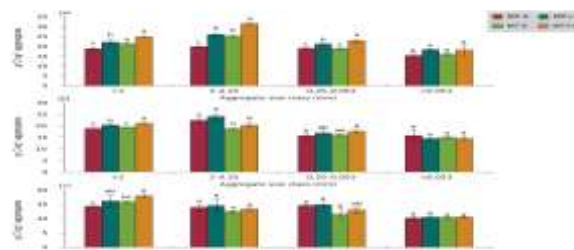
\* Data are represented as means ± S.D., and data with the same letters within each column indicate no significant difference at  $P = 0.05$  level.

Naresh et al. (2015a) also found that conservation tillage practices significantly influenced the total soil carbon (TC), total inorganic carbon (TIC), total soil organic carbon (SOC) and oxidizable organic carbon (OC) content of the surface (0 to 15 cm) soil. Wide raised beds transplanted rice and zero till wheat with 100% ( $T_9$ ) or with 50% residue retention ( $T_8$ ) showed significantly higher TC, SOC content of 11.93 and 10.73  $\text{g kg}^{-1}$  in  $T_9$  and 10.98 and 9.38  $\text{g kg}^{-1}$ , respectively in  $T_8$  as compared to the other treatments. Irrespective of residue incorporation/ retention, wide raised beds with zero till wheat enhanced 40.5, 34.5, 36.7 and 34.6% of TIC, TC, SOC and OC in surface soil as compared to CT with transplanted rice cultivation. Aulakh *et al.* (2013) showed that PMN content after 2 years of the experiment in 0-5 cm soil layer of CT system,  $T_2$ ,  $T_3$  and  $T_4$  treatments increased PMN content from 2.7  $\text{mg kg}^{-1} 7\text{d}^{-1}$  in control ( $T_1$ ) to 2.9, 3.9 and 5.1  $\text{mg kg}^{-1} 7\text{d}^{-1}$  without CR, and to 6.9, 8.4 and 9.7  $\text{mg kg}^{-1} 7\text{d}^{-1}$  with CR ( $T_6$ ,  $T_7$  and  $T_8$ ), respectively. The corresponding increase of PMN content under CA system was from 3.6  $\text{mg kg}^{-1} 7\text{d}^{-1}$  in control to 3.9, 5.1 and 6.5  $\text{mg kg}^{-1} 7\text{d}^{-1}$  without CR and to 8.9, 10.3 and 12.1  $\text{mg kg}^{-1} 7\text{d}^{-1}$  with CR. PMN, a measure of the soil capacity to supply mineral N, constitutes an important measure of the soil health due to its strong relationship with the capability of soil to supply N for crop growth.

Ou et al. (2016) reported that the tillage systems obviously affected the distribution of soil aggregates with different sizes [Fig.8a]. The proportion of the  $>2$  mm aggregate fraction in NT+S was 7.1 % higher than that in NT-S in the 0.00-0.05 m layer. There was no significant difference in the total amount of all the aggregate fractions between NT+S and NT-S in both the 0.05-0.20 and 0.20-0.30 m layers. NT+S and NT-S showed higher proportions of  $>2$  mm aggregate and lower proportions of  $<0.053$  mm aggregate compared to the MP system for the 0.00-0.20 m layer. The proportion of  $>0.25$  mm macro-aggregate was significantly higher in MP+S than in MP-S in most cases, but the proportion of  $<0.053$  mm aggregate was 11.5-20.5 % lower in MP+S than in MP-S for all the soil layers [Fig. 8a]. Du et al. (2013) reported that the NT system did affect the SOC stock distribution in the soil profile but not the total quantity. Tillage regimes obviously influenced soil aggregation distribution in the soil profile. In the upper 0.00-0.05 and 0.05-0.20 m layers, the NT system improved the formation level of the  $>2$  mm aggregate but reduced the formation level of  $<0.053$  mm aggregates, compared to the MP system, suggesting that mechanical operation reduced large-macro-aggregate formation and disrupted soil macro-aggregates into individual particles (Huang et al., 2010 and Jiang et al., 2011). The aggregate-associated SOC concentration in different soil layers was influenced by tillage systems [Fig.8b]. In the 0.00-0.05 m layer, SOC concentration in macro-aggregates showed the order of NT+S  $>$  MP+S = NT-S  $>$  MP-S, whereas the NT system was superior to the MP system. However, the NT system significantly reduced the SOC concentration in the 2.00-0.25 mm fraction in the 0.05-0.20 m layer. A similar trend was observed in the 0.25-0.053 mm fraction in the 0.20-0.30 m layer. Across all the soil layers, there was no difference in the  $<0.053$  mm fraction between NT-S and MP-S, as well as between NT+S and MP+S, indicating that the NT system did not affect the SOC concentration in the silt + clay fraction. In average across the soil layers, the SOC concentration in the macro-aggregate was increased by 13.5 % in MP+S, 4.4 % in NT-S and 19.3 % in NT+S, and those in the micro-aggregate ( $<0.25$  mm) were increased by 6.1 % in MP+S and 7.0 % in NT+S compared to MP-S. For all the soil layers, the SOC concentration in all the aggregate size classes was increased with straw incorporation, by 20.0, 3.8 and 5.7 % under the MP system, and 20.2, 6.3 and 8.8 % under the NT system [Fig. 8b]. The higher proportion of  $>2$  mm aggregates and lower proportion of  $<0.053$  mm aggregates under NT systems might be the result of the higher soil hydrophobicity, low intensity of wetting and drying cycles, higher soil C concentration or the physical and chemical characteristics of large macro-aggregates making them more resistant to breaking up (Vogelmann et al., 2013).



**FIG.8 (a): Distribution (%) of water-stable aggregates with different sizes in different soil layers as influenced by tillage treatments. (a) 0.00-0.05 m; (b) 0.05-0.20 m; (c) 0.20-0.30 m. MP-S: moldboard plow without straw; MP+S: moldboard plow with straw; NT-S: no-tillage without straw; NT+S: no-tillage with straw [Source: Ou et al., 2016].**



**FIG.8 (b): Aggregate-associated SOC concentration in different layer intervals as influenced by tillage treatments. (a) 0.00-0.05 m; (b) 0.05-0.20 m; (c) 0.20-0.30 m. MP-S: moldboard plow without straw; MP+S: moldboard plow with straw; NT-S: no-tillage without straw; NT+S: no-tillage with straw.**

Guo et al. (2016) also found that compared with CT treatments, NT treatments did not affect SOC concentration of bulk soil in the 5–20 cm soil layer, but significantly increased the SOC concentration of bulk soil in the 0–5 cm soil layer [Table 5]. In comparison with NS treatments, S treatments had not significant effects on SOC concentration of bulk soil in the 5–20 cm soil layer, but significantly enhanced the SOC concentration of bulk soil in the 0–5 cm soil layer [Table 5]. In the 0–5 cm soil layer, NT treatments significantly increased SOC concentration by 5.8%, 6.8%, and 7.9% of bulk soil, >0.25 mm aggregate, and <0.25 mm aggregate, respectively, compared with CT treatments [Table 5]. NT treatments significantly increased MBC of bulk soil, >0.25 mm and <0.25 mm aggregates by 11.2%, 11.5% and 20.0%, respectively, compared with CT treatments. DOC concentrations of bulk soil, >0.25 mm aggregate, and <0.25 mm aggregate under NT treatments were 15.5%, 29.5%, and 14.1% higher than those under CT treatments, respectively. In comparison with NS treatments, S treatments significantly increased SOC concentrations of bulk soil by 12.8%, >0.25 mm aggregate by 11.3%, and <0.25 mm aggregate by 14.1%. In addition, MBC of bulk soil, >0.25 mm aggregate, and <0.25 mm aggregate under S treatments were 29.8%, 30.2%, and 24.1% higher than those of NS treatments, respectively. S treatments exhibited 25.0%, 37.5%, and 23.2% higher DOC concentrations of bulk soil, >0.25 mm aggregate, and <0.25 mm aggregate compared with NS treatments, respectively. In the 0–5 cm soil layer, there were significant interactions of tillage and straw returning on SOC concentration of >0.25 mm and <0.25 mm aggregates, MBC of bulk soil and <0.25 mm aggregate, and DOC concentration of >0.25 mm aggregate [Table 5]. This increase in SOC concentration can be attributed to a combination of less soil disturbance and more residues returned to the soil surface under conservation tillage (Dikgwatlhe et al., 2014).

**TABLE 5**  
**CHANGES IN SOC FRACTIONS WITHIN AGGREGATES UNDER DIFFERENT TILLAGE AND RESIDUE TREATMENTS [GUO ET AL., 2016].**

Organic C	Soil fractions	CTNS	CTS	NTNS	NTS
SOC (0–5 cm soil layer) (g kg <sup>-1</sup> )	Bulk soil	19.60±0.55 d	21.29±0.12 b	20.33±0.46 c	21.75±0.18 a
	>0.25 mm	19.70±0.10 c	21.30±0.10 b	20.43±0.06 c	23.37±0.06 a
	<0.25 mm	17.28±0.06 d	19.48±0.12 b	18.41±0.17 c	21.24±0.18 a
SOC (5–10 cm soil layer) (g kg <sup>-1</sup> )	Bulk soil	17.84±0.56 a	18.10±0.20 a	17.87±0.87 a	18.31±0.17 a
	>0.25 mm	/	/	/	/
	<0.25 mm	/	/	/	/
SOC (10–20 cm soil layer) (g kg <sup>-1</sup> )	Bulk soil	15.67±0.47 a	15.97±0.41 a	15.53±0.41 a	15.50±0.20 a
	>0.25 mm	/	/	/	/
	<0.25 mm	/	/	/	/
MBC (0–5 cm soil layer) (mg kg <sup>-1</sup> )	Bulk soil	1846±5.84 d	2366±38.58 b	2024±11.40 c	2657±28.71 a
	>0.25 mm	1962±3.68 d	2538±27.09 b	2173±57.73 c	2844±22.90 a
	<0.25 mm	1517±10.56 c	1820±14.42 b	1758±11.33 b	2245±33.86 a
DOC (0–5 cm soil layer) (g kg <sup>-1</sup> )	Bulk soil	1.09±0.04 d	1.33±0.03 b	1.22±0.03 c	1.56±0.04 a
	>0.25 mm	1.05±0.05 d	1.43±0.03 b	1.34±0.01 c	1.86±0.01 a
	<0.25 mm	0.89±0.03 d	1.10±0.02 b	1.01±0.02 c	1.25±0.02 a

Different letters in a line denote significant differences among treatments.

CTNS, conventional intensive tillage with straw removal; CTS, conventional intensive tillage with straw returning; NTNS, no-tillage with straw removal; tillage; NTS, no-tillage with straw returning. SOC, soil organic C; MBC, microbial biomass C; DOC, dissolved organic C

Naresh et al. (2018) reported that the SOC pool was the highest in the 100 per cent RDF + VC (56.8 Mg C ha<sup>-1</sup>), and it was on par with 50 per cent RDF + VC (52.8 Mg C ha<sup>-1</sup>) > 75 per cent RDF + VC (51.4 Mg C ha<sup>-1</sup>) > VC (49.4 Mg C ha<sup>-1</sup>) > RDF (39.3 Mg C ha<sup>-1</sup>) > control (35.9 Mg C ha<sup>-1</sup>) treatments. A higher percentage of C build-up was observed in 100 per cent RDF + VC treatment (43.6 per cent) followed by 50 per cent RDF + VC treatment (40.7 per cent), which was reflected in the profile SOC concentration of respective treatments. The SOC build-up rate also followed a similar trend as C build-up. The C budgeting shows that 36.8 per cent of the C applied as VC was stabilized. With the exception of the control and sole application of RDF through chemical fertilizer, the magnitude of SOC sequestration in other treatments was 7.9–9.6 Mg ha<sup>-1</sup>. Higher SOC sequestration was observed with the application of vermicompost along with 100, 75 and 50 per cent recommended rate of RDF. Cultivation of a crop without using any organic and/or inorganic fertilizer inputs (control) caused a net depletion of SOC pool by 12.0 Mg C ha<sup>-1</sup>. Though application of VC decreased the bulk density of the soil particularly at surface and subsurface layer due to higher SOC and increased root biomass it improves the SOC concentration significantly and ultimately increased SOC stock of the profile. SOC concentrations and stocks increased considerably with organic manure incorporation rates, which are possibly attributed to a larger proportion of recalcitrant organic compounds in manure (Liu *et al.*, 2014). Vermi-compost manure application can result in an increase in lignin and lignin-like products, which are major components of the resistant C pool in the soil (Lima *et al.*, 2009). Crop production was also enhanced by the manure inputs,

which lead to higher total C inputs from rhizodeposition, root biomass and stubble return [Table 6]. The prevailing low levels of SOC concentrations are attributed to soil-mining practices – a little or no crop residues returned to the soil, excessive tillage, unbalanced fertilizer use and severe soil degradation. Ploughing for seedbed preparation disturbs the soil, adversely affects the distribution and stability of aggregates, exacerbates the oxidation of SOM and depletes the SOC pool (Kong *et al.*, 2005).

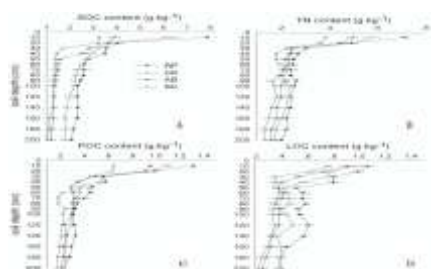
**TABLE 6**  
**PROFILE ORGANIC C (OC), C BUILD-UP, C BUILD-UP RATE, C SEQUESTERED, C: N RATIO AND WET AGGREGATE STABILITY (WAS) IN THE SOIL PROFILE AS AFFECTED BY 7 YR OF TILLAGE CROP RESIDUE AND NUTRIENT MANAGEMENT PRACTICES [SOURCE: NARESH ET AL., 2018]**

Treatments	Profile OC Mg ha <sup>-1</sup>	C build-up %	C build-up rate Mg C ha <sup>-1</sup> y <sup>-1</sup>	C Sequestered Mg C ha <sup>-1</sup>	C:N Ratio	WAS (%)
<b>Tillage crop residue practices</b>						
T <sub>1</sub>	43.5±3.1 <sup>d</sup>	27.9±0.7 <sup>c</sup>	1.06±0.08 <sup>a</sup>	6.7±0.2 <sup>d</sup>	14.5 <sup>ab</sup>	93.4 <sup>c</sup>
T <sub>2</sub>	51.7±2.5 <sup>c</sup>	34.2±1.8 <sup>b</sup>	1.36±0.07 <sup>cd</sup>	8.2±0.1 <sup>c</sup>	12.7 <sup>b</sup>	92.9 <sup>c</sup>
T <sub>3</sub>	69.4±3.3 <sup>a</sup>	36.6±0.6 <sup>b</sup>	1.46±0.09 <sup>b</sup>	8.6±0.8 <sup>bc</sup>	9.43 <sup>c</sup>	95.7 <sup>ab</sup>
T <sub>4</sub>	63.3±2.8 <sup>b</sup>	31.8±0.6 <sup>bc</sup>	1.33±0.04 <sup>d</sup>	7.6±0.8 <sup>b</sup>	13.5 <sup>ab</sup>	94.5 <sup>bc</sup>
T <sub>5</sub>	72.9±3.7 <sup>a</sup>	41.0±2.2 <sup>a</sup>	1.63±0.09 <sup>a</sup>	9.2±0.2 <sup>ab</sup>	12.3 <sup>b</sup>	96.9 <sup>a</sup>
T <sub>6</sub>	73.0±3.6 <sup>a</sup>	41.2±2.3 <sup>a</sup>	1.64±0.10 <sup>a</sup>	9.6±0.2 <sup>a</sup>	9.28 <sup>c</sup>	97.6 <sup>a</sup>
T <sub>7</sub>	41.5±2.9 <sup>d</sup>	22.4±1.2 <sup>c</sup>	0.89±0.06 <sup>f</sup>	5.3±0.5 <sup>e</sup>	15.3 <sup>a</sup>	89.7 <sup>d</sup>
<b>Fertilizer Management Practices</b>						
F <sub>1</sub>	35.9±1.6 <sup>c</sup>	-	-	-12.0±0.7 <sup>d</sup>	16.2 <sup>a</sup>	93.7 <sup>d</sup>
F <sub>2</sub>	39.3±1.8 <sup>c</sup>	29.8±0.06 <sup>d</sup>	1.28±0.007 <sup>d</sup>	-0.61±0.8 <sup>c</sup>	15.3 <sup>ab</sup>	92.3 <sup>bc</sup>
F <sub>3</sub>	52.8±0.02 <sup>ab</sup>	40.7±2.4 <sup>a</sup>	1.82±0.006 <sup>a</sup>	9.3±0.8 <sup>a</sup>	14.5 <sup>bc</sup>	90.1 <sup>b</sup>
F <sub>4</sub>	51.4±2.1 <sup>ab</sup>	37.3±0.06 <sup>b</sup>	1.73±0.021 <sup>b</sup>	8.5±0.5 <sup>b</sup>	13.7 <sup>c</sup>	89.9 <sup>b</sup>
F <sub>5</sub>	56.8±1.9 <sup>c</sup>	43.6±0.09 <sup>a</sup>	1.88±0.001 <sup>a</sup>	9.6±0.7 <sup>a</sup>	8.99 <sup>a</sup>	87.4 <sup>a</sup>
F <sub>6</sub>	49.4±2.3 <sup>b</sup>	34.2±1.8 <sup>c</sup>	1.46±0.07 <sup>c</sup>	7.9±0.3 <sup>c</sup>	10.8 <sup>d</sup>	91.1 <sup>bc</sup>

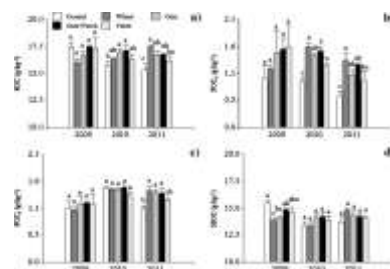
Zhao *et al.* (2014) concluded that the contents of SOC, TN, POC and LOC responded differently as the change of soil depth [Fig.9a]. In all land use types, contents of SOC, TN, POC and LOC in top soil (0–10 cm) were 3.26–7.86 g.kg<sup>-1</sup>, 0.39–0.72 g.kg<sup>-1</sup>, 0.65–1.31 g.kg<sup>-1</sup> and 0.76–1.07 g.kg<sup>-1</sup>, respectively, which were significantly higher than other soil layers. The contents of SOC, TN, POC and LOC decreased significantly in soil depth of 10–40 cm while the decreases trended to be flatter in subsoil (40–100 cm). Additionally, the differences in contents of SOC, TN, POC and LOC in deep subsoil (100–200 cm) were negligible. Vegetation can greatly influence soil quality, C and N cycling, and regional socioeconomic development (Fu *et al.*, 2010). It is also reported that converting cropland into land with perennial vegetation would increase the SOC content (Groenendijk *et al.*, 2002).

Duval *et al.* (2016) reported that the concentration of labile soil organic carbon (POC<sub>c</sub> and POC<sub>f</sub>) did not reflect any differences between the cover crops and Ct in 2008 at 0–20 cm [Fig.9b]. The 3 years of C-input by cover crops were insufficient to affect the most dynamic and labile fractions of SOM, despite C-input differences among treatments. As from 2009, SOC increase by the cover crops was mainly due to higher POC<sub>c</sub> concentration [Fig.9b]. In 2009 and 2011, the cover crops significantly enhanced POC<sub>c</sub> levels compared with Ct. Differences among cover crops were also found. In general, gramineous species showed higher POC<sub>c</sub> concentration than V. This difference among species may have been caused by the higher quality of the legume contribution (lower C: N), which stimulated residue decomposition and thus had a direct influence on POC<sub>c</sub>. Regarding the Ct treatment, POC<sub>c</sub> increased in the W, O and O + V treatments by 66 and 95% in 2009 - 2011, respectively, whereas in the V treatment, POC<sub>c</sub> rose by 33 and 49% for the same periods [Fig.9b]. These results suggest that cover crops of gramineous species would enhance accumulation of more recalcitrant materials on the soil surface, thus promoting SOM increase. Also, larger residue amounts with a high concentration of soluble compounds and a low C: N ratio (vetch + soybean residues) would fuel microbial activity, stimulate decomposition and have a negative effect on organic fractions (Scherer-Lorenzen, 2008).

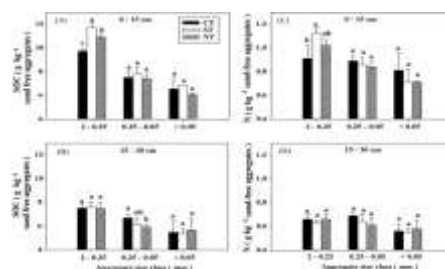
Chen *et al.* (2009) also found that the amount of large macro-aggregates was extremely low and made up of almost all rocks, therefore SOC and Nt content was not determined in large macro-aggregates. The influence of tillage on aggregate C and Nt content is shown in [Fig.9c]. At 0–15 cm, tillage effect was confined to the 2–0.25 mm size fraction, in which the conservation tillage treatments contained significantly higher SOC contents than CT, ST had significantly higher Nt contents than CT, and NT tended to have higher Nt contents than CT [Fig.9c]. No significant differences were detected in SOC and Nt contents in the 0.25– 0.05 mm and <0.05 mm classes among all treatments [Fig.9c].



**FIG. 9(a):** Distribution of soil organic carbon (SOC, A), total nitrogen (TN, B), particulate organic carbon (POC, C), and labile organic carbon (LOC, D) contents of different land used types in soil depth of 0–200 cm [Source: Zhao et al., 2014].



**FIG. 9(b):** Total organic carbon (SOC) (a), coarse particulate organic carbon (POCc) (b), fine particulate organic carbon (POCf) (c) and mineral-associated organic carbon (MOC) (d) as affected by cover crops at 0–20 cm depth [Source: Duval et al., 2016].



**FIG. 9(c):** Soil organic carbon (SOC) and nitrogen content (g kg<sup>-1</sup>) of sand-free aggregates from two depths under conventional tillage with residue removal (CT), shallow tillage with residue cover (ST), and no-tillage with residue cover (NT) [Chen et al., 2009].

Krishna et al. (2018) reported that the total organic carbon (TOC) allocated into different pools in order of very labile > less labile > non labile >labile, constituting about 41.4, 20.6, 19.3 and 18.7%, respectively. In comparison with control, system receiving farmyard manure (FYM-10 Mg ha<sup>-1</sup> season<sup>-1</sup>) alone showed greater C build up (40.5%) followed by 100% NPK+FYM (120:60:40 kg N, P, K ha<sup>-1</sup>+ 5 Mg FYM ha<sup>-1</sup>season<sup>-1</sup>) (16.2%). In fact, a net depletion of carbon stock was observed with 50% NPK (-1.2 Mg ha<sup>-1</sup>) and control (-1.8 Mg ha<sup>-1</sup>) treatments. Only 28.9% of C applied through FYM was stabilized as SOC. A minimal input of 2.34 Mg C ha<sup>-1</sup> y<sup>-1</sup> is needed to maintain SOC level [Table 7]. The magnitude of carbon pools extracted under a gradient of oxidizing conditions was as follows: C<sub>VL</sub>>C<sub>LL</sub> > C<sub>NL</sub> > C<sub>L</sub> constituting about 41.4, 20.6, and 19.3 and 18.7%, respectively, of the TOC [Table 7]. However, the contribution of VL, L and LL pools to SOC was 51.2, 23.1 and 25.5%, respectively. While active pool (C<sub>VL</sub> + C<sub>L</sub>) constituted about 60.1%, passive pool (C<sub>LL</sub> + C<sub>NL</sub>) represented 39.9% of the TOC. Among the treatments, 100% NPK+FYM (44.4%) maintained a proportionately higher amount of soil C in passive pools. With an increase in the dose of fertilization, on average, C allocation into passive pool was increased (33.0, 35.3, 40.7% and 39.3% of TOC under control, 50% NPK, 100% NPK and 150% NPK treatments, respectively).

**TABLE 7**

**OXIDISABLE ORGANIC CARBON FRACTIONS (VERY LABILE, LABILE, LESS LABILE AND NON-LABILE) IN SOILS (G KG-1) AT DIFFERENT LAYERS (CM) [KRISHNA ET AL., 2018]**

Treatment	Very labile C				Labile C			
	0-15	15-30	30-45	Total	0-15	15-30	30-45	Total
Control	3.6±0.5 <sup>c</sup>	1.4±0.3 <sup>b</sup>	1.3±0.2 <sup>a</sup>	6.3±0.4 <sup>b</sup>	2.4±0.3 <sup>a</sup>	1.0±0.2 <sup>a</sup>	0.8±0.4 <sup>a</sup>	4.2±0.6 <sup>a</sup>
50% NPK	4.6±0.3 <sup>bc</sup>	2.1±0.7 <sup>ab</sup>	1.5±0.1 <sup>a</sup>	8.1±0.9 <sup>a</sup>	1.7±0.4 <sup>ab</sup>	0.9±0.5 <sup>a</sup>	0.7±0.2 <sup>a</sup>	3.3±0.7 <sup>a</sup>
100% NPK	4.4±0.3 <sup>bc</sup>	2.3±0.2 <sup>a</sup>	1.4±0.5 <sup>a</sup>	8.0±0.7 <sup>a</sup>	1.8±0.4 <sup>ab</sup>	0.8±0.5 <sup>a</sup>	0.6±0.3 <sup>a</sup>	3.2±0.8 <sup>a</sup>
150% NPK	5.0±0.2 <sup>ab</sup>	2.6±0.2 <sup>a</sup>	1.5±0.1 <sup>a</sup>	9.0±0.3 <sup>a</sup>	1.2±0.3 <sup>b</sup>	0.7±0.2 <sup>a</sup>	0.9±0.2 <sup>a</sup>	2.8±0.4 <sup>a</sup>
100% NPK+FYM	4.8±0.2 <sup>ab</sup>	2.0±0.2 <sup>ab</sup>	1.3±0.3 <sup>a</sup>	8.1±0.2 <sup>a</sup>	1.9±0.3 <sup>ab</sup>	0.7±0.2 <sup>a</sup>	0.7±0.3 <sup>a</sup>	3.4±0.2 <sup>a</sup>
FYM	5.9±1.3 <sup>a</sup>	2.2±0.2 <sup>a</sup>	1.4±0.3 <sup>a</sup>	9.5±1.6 <sup>a</sup>	2.5±0.9 <sup>a</sup>	0.7±0.3 <sup>a</sup>	0.7±0.2 <sup>a</sup>	3.9±0.9 <sup>a</sup>
Fallow	4.2±0.7 <sup>bc</sup>	1.5±0.5 <sup>b</sup>	0.7±0.3 <sup>b</sup>	6.3±0.8 <sup>b</sup>	2.2±1.0 <sup>ab</sup>	1.0±0.3 <sup>a</sup>	1.0±0.4 <sup>a</sup>	4.1±1.1 <sup>a</sup>
	Less labile C				Non labile C			
Control	1.5±0.3 <sup>c</sup>	0.6±0.4 <sup>c</sup>	0.4±0.0 <sup>c</sup>	2.6±0.7 <sup>d</sup>	1.2±0.5 <sup>b</sup>	1.2±0.3 <sup>a</sup>	0.2±0.2 <sup>b</sup>	2.6±0.5 <sup>b</sup>
50% NPK	1.8±0.1 <sup>c</sup>	0.4±0.1 <sup>c</sup>	0.5±0.2 <sup>c</sup>	2.7±0.1 <sup>cd</sup>	1.2±0.9 <sup>b</sup>	1.7±0.8 <sup>a</sup>	0.7±0.4 <sup>ab</sup>	3.5±1.8 <sup>ab</sup>
100% NPK	2.5±0.3 <sup>ab</sup>	0.8±0.1 <sup>bc</sup>	1.1±0.2 <sup>ab</sup>	4.4±0.1 <sup>b</sup>	1.3±0.6 <sup>b</sup>	1.5±0.6 <sup>a</sup>	0.5±0.2 <sup>ab</sup>	3.3±1.0 <sup>ab</sup>
150% NPK	2.6±0.2 <sup>a</sup>	0.9±0.1 <sup>bc</sup>	0.4±0.2 <sup>c</sup>	3.9±0.1 <sup>b</sup>	1.4±0.3 <sup>b</sup>	1.5±0.2 <sup>a</sup>	0.8±0.1 <sup>a</sup>	3.7±0.3 <sup>ab</sup>
100% NPK+FYM	2.7±0.6 <sup>a</sup>	1.5±0.2 <sup>a</sup>	1.4±0.1 <sup>a</sup>	5.6±0.7 <sup>a</sup>	2.0±0.8 <sup>b</sup>	1.3±0.1 <sup>a</sup>	0.3±0.3 <sup>ab</sup>	3.5±0.7 <sup>ab</sup>
FYM	1.9±0.7 <sup>bc</sup>	1.7±0.2 <sup>a</sup>	1.0±0.2 <sup>b</sup>	4.5±0.7 <sup>ab</sup>	3.7±1.3 <sup>a</sup>	1.0±0.2 <sup>a</sup>	0.5±0.5 <sup>ab</sup>	5.1±1.9 <sup>a</sup>
Fallow	1.5±0.3 <sup>c</sup>	1.3±0.7 <sup>ab</sup>	0.9±0.4 <sup>b</sup>	3.8±1.2 <sup>bc</sup>	2.1±0.2 <sup>b</sup>	1.4±0.7 <sup>a</sup>	0.4±0.2 <sup>ab</sup>	3.9±0.9 <sup>ab</sup>

\*values in the same column followed by different letters are significantly different at P<0.001 according to Duncan's Multiple Range Test (DMRT) for separation of means. ± indicates the standard deviation values.

Nath et al. (2015) revealed that the TOC content for all the treatments was high in surface soil (0-10 cm) than in subsurface soil (10- 30 cm). TOC in surface and sub-surface soil was in the order organic > organic + inorganic > VM >inorganic > control and organic > organic +inorganic > inorganic > VM > control respectively [Table 8]. Build-up of higher amount of TOC in surface soil over sub-surface soil is attributed to accumulation of organic matter from root biomass and left over crop residues in the former that decreased with soil depth. Addition of root biomass and root exudates results in such variation in soil depths (Kaur et al., 2008). Application of organic manure alone or in combination with inorganic fertilizer considerably

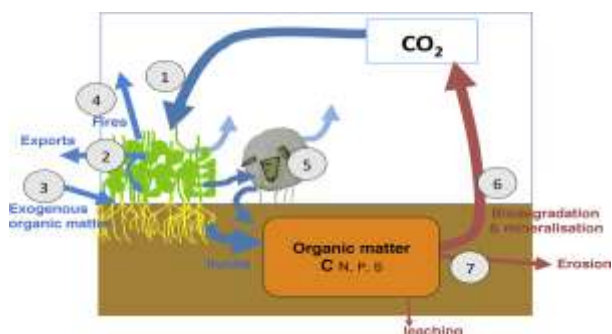
increased TOC in 0-10 cm soil depth than control plot [Table 8]. A higher percentage of C build-up was observed in 100 per cent RDF + VC treatment (43.6 per cent) followed by 50 per cent RDF+VC treatment (40.7 per cent), which was reflected in the profile SOC concentration of respective treatments [Table 8]. The SOC build-up rate also followed a similar trend as C build-up. The C budgeting shows that 36.8 per cent of the C applied as VC was stabilized. Higher SOC sequestration was observed with the application of vermin-compost along with 100, 75 and 50 per cent recommended rate of RDF. Cultivation of a crop without using any organic and/or inorganic fertilizer inputs (control) caused a net depletion of SOC pool by 12.0 Mg C ha<sup>-1</sup>. Maintaining the SOC pool above the critical level is necessary to sustain agronomic productivity and to minimize environmental degradation (Lal, 2010c). However, maintaining or improving the SOC pool in light-textured soils of arid and semi-arid regions is a major challenge (Srinivasarao *et al.*, 2012).

**TABLE 8**  
**SOIL ORGANIC CARBON (SOC) POOLS UNDER DIFFERENT MANAGEMENT REGIMES IN SURFACE SOIL (0-10 CM) AND SUBSURFACE (10-30 CM) PADDY GROWING SOILS [NATH ET AL., 2015]**

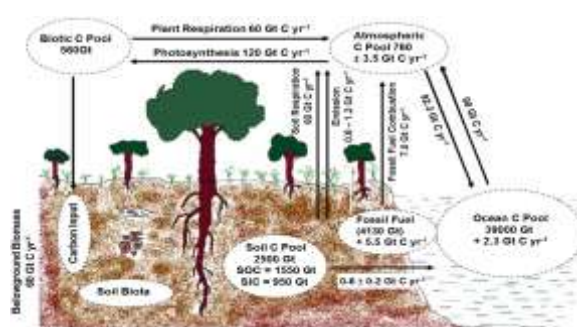
Treatments	Sub fractionation of organic carbon (%)				TOC (%)	Active pool (C <sub>ap</sub> )	Passive pool (C <sub>pp</sub> )
	Very labile (C <sub>vl</sub> )	Labile (C <sub>l</sub> )	Less labile (C <sub>ll</sub> )	Non-labile (C <sub>nl</sub> )			
<b>0-10 cm</b>							
Control	0.28 (22%)	0.04 (3%)	0.10 (8%)	0.88 (67%)	1.30 <sup>a</sup>	25%	75%
VM	0.33 (24%)	0.10 (7%)	0.17 (12%)	0.76 (57%)	1.36 <sup>b</sup>	31%	69%
Inorganic	0.30 (23%)	0.10 (8%)	0.14 (11%)	0.79 (59%)	1.33 <sup>a</sup>	30%	70%
Organic	0.36 (25%)	0.13 (9%)	0.12 (8%)	0.85 (59%)	1.46 <sup>b</sup>	34%	66%
Organic+Inorganic	0.37 (26%)	0.14 (10%)	0.05 (4%)	0.87 (60%)	1.43 <sup>b</sup>	36%	64%
<b>10-30 cm</b>							
Control	0.13 (19%)	0.06 (9%)	0.16 (23%)	0.35 (50%)	0.70 <sup>a</sup>	27%	73%
VM	0.15 (19%)	0.10 (13%)	0.15 (20%)	0.40 (49%)	0.80 <sup>b</sup>	31%	69%
Inorganic	0.13 (16%)	0.11 (14%)	0.17 (21%)	0.40 (49%)	0.81 <sup>b</sup>	30%	70%
Organic	0.14 (19%)	0.09 (12%)	0.10 (14%)	0.41 (55%)	0.74 <sup>b</sup>	31%	69%
Organic+Inorganic	0.16 (19%)	0.09 (11%)	0.15 (18%)	0.45 (53%)	0.85 <sup>b</sup>	29%	71%
<b>0-30 cm</b>							
Control	0.21 (21%)	0.05 (5%)	0.13 (13%)	0.61 (61%)	1.0 <sup>a</sup>	26%	74%
VM	0.24 (22%)	0.10 (9%)	0.16 (15%)	0.58 (54%)	1.08 <sup>b</sup>	31%	69%
Inorganic	0.22 (20%)	0.11 (10%)	0.16 (14%)	0.60 (56%)	1.07 <sup>b</sup>	30%	70%
Organic	0.25 (23%)	0.11 (10%)	0.11 (10%)	0.63 (57%)	1.24 <sup>b</sup>	33%	67%
Organic+Inorganic	0.27 (23%)	0.12 (10%)	0.10 (9%)	0.66 (58%)	1.14 <sup>b</sup>	33%	67%

Parenteses show percent of TOC; different letters superscripted refers to significant differences between the treatments at 5% level of significance. [Control: without any organic and inorganic fertilizer; VM: village management (partially decomposed cow dung applied @ 70-80 Mg ha<sup>-2</sup>); Inorganic (NPK) fertilizer (130-100-60 was used in the form of urea, single superphosphate and muriate of potash); Organic manure (phosphate solubilizing biofertilizer and azobacter bio-fertilizer applied in two steps: seedlings dip and soil application; Organic+Inorganic: both organic and inorganic fertilizer applied together].

Application of high fertilizer N rate in high C: N residue amended soils lowers the C: N ratio of the residue which avoids net immobilization but enhances the mineralization process (Pathak *et al.*, 2006). This N remained in the soil after harvest and helped to maintain inorganic N concentrations in soil. The WAS under ZT without residue retention (93.4%) significantly increased by 4% compared to CT system (89.7%). A similar trend was observed under fertilizer management practices where control (91.7%) significantly increased WAS by 2.3% compare to 100% VC (93.9%). The 50%RDF +50%VC and 75%RDF +25%VC decreased WAS by 4% compared to under 100% RDF system [Table 8]. The study reported that mechanical tillage increased the breakdown of soil macro-aggregates and that CT disrupted soil macro-aggregates into micro-aggregates or individual particles. In addition, soil under CT system distributed aggregates during the plowing event by bringing protected aggregates to the soil surface.



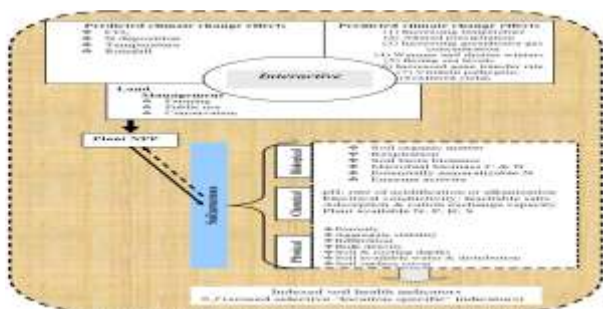
**FIG. 10(a): Levers associated with agricultural practices that may influence SOC stocks.**



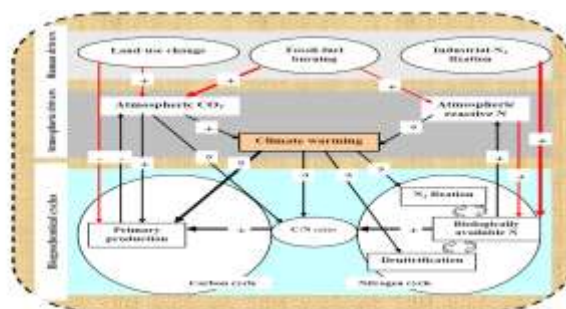
**FIG. 10(b): Sources and sinks of carbon from different pools under terrestrial and aquatic ecosystems.**

Several organic cropping systems, characterized by a diversified rotation including legume cover crops, exhibited similar or higher SOC stocks than their conventional counterparts, while fresh OC inputs to soil were not higher and tillage was more frequent (Autret et al., 2016) (a process not represented in [Fig. 10a]. Kallenbach et al. (2015) showed that in an organic cropping system, soil microorganisms had a higher carbon use efficiency and higher growth rates than under the reference conventional system. This should result in more microbial necromass being formed per unit of C input. Microbial necromass represents a significant fraction of soil organic matter and a major constituent of SOM stabilized in the long term (Cotrufo et al., 2013), which would explain the increased or preserved SOC stocks.

Each year, an estimated 25–40 billion tons of fertile soil are lost globally (FAO and ITPS, 2015). Hence, improving soil health through sustainable land management should be a common goal for farmers and land managers, to protect, maintain and build their most vital resource – soils. Soils are the major reservoir of C in terrestrial ecosystems, and soil C plays a dynamic role in influencing the global C cycle and climate change [Fig. 10b] while regulating soil health and productivity (Mehra et al., 2018; Singh et al., 2018). Soil contains C in two forms: soil organic C (SOC) and soil inorganic C (SIC), with most soils (except calcareous soils) having more SOC than SIC [Fig. 10b]. Thus far, enormous scientific progress has been attained in understanding soil functional characteristics relating to SOC stocks and C dynamics in agro-ecosystems (Stockmann et al., 2013).



**FIG. 11(a):** Potential links between climate change, land use and management change, and soil health indicators (Source: Allen et al. 2011)



**FIG.11 (b):** Potential nitrogen-carbon-climate interactions (Source: Gruber and Galloway, 2008)

Allen et al. (2011) revealed that climate change scenarios considered by Intergovernmental Panel on Climate Change (IPCC) prediction include increase in atmospheric CO<sub>2</sub> concentration, increases in air temperature, changes in precipitation and prevalence of extreme climate events. For instance, global temperature change of 1.6–6.4 °C by 2100, atmospheric CO<sub>2</sub> concentration increases by up to 550 ppm and precipitation change by at least 20% have been predicted (IPCC, 2007a). However, the predicted changes vary geographically and with future greenhouse gas (GHG) emission control. Therefore, the actual magnitude of changes of these parameters and consequences of these changes will therefore be location specific and be dependent on the extent of future success in reducing emission of GHG. Changes in precipitation are likely to be different for different parts of the world [Fig.11a].

Gruber and Galloway, (2008) also found that, soil organic matter (SOM) is essential in maintaining physical, chemical and biological functions in soil. In fact, SOM is the key indicator of soil health. It contains both living and non-living components. Living components include soil microbial biomass and living roots. Non-living SOM is a heterogeneous organic matter, variously described as labile, slow and recalcitrant SOM, light fraction (free or occluded) and heavy fraction, particulate (> 53 μm) and non-particulate SOM. It is also described by its chemical constituents such as proteins, lipids, starch, carbohydrates, hemicelluloses, celluloses, lignins, polyphenols, pectins and tannins or by humic acid, fulvic acid and humins. Soil organic carbon (SOC) constitutes about 50% of SOM and contains labile, slow and recalcitrant C pools. It could be also considered that, the influence of atmospheric N deposition, an important component of global environmental change; the rates of N deposition have increased by threefold to fivefold over the past century and may continue to increase rapidly in densely populated areas. The increasing rates of atmospheric N deposition may play a major role in modulating climate change impacts [Fig.11b].

### III. CONCLUSION

In topsoil, WS macro-aggregate formation was highest (28.2 g of >250 μm aggregates per gram of C added) with the lowest residue input (2.5 g residue-C kg<sup>-1</sup> soil). In the subsoil, WS macro-aggregate formation increased to 76.3 g of >250 μm



aggregates per gram of C added with residue input of 5 g residue-C kg<sup>-1</sup> soil and decreased thereafter. The concentration of POC, MBC and HWC were higher under topsoil (0-10 cm) as compared to subsoil (10-20 cm) in CA practices. Organic carbon concentrations in the <0.053-, 0.053- to 0.25-, 0.25- to 2.0, and >2.0-mm fractions were 14.0, 12.0, 14.4, 24.1% greater, respectively, in CA than in CF. The contents of SOC, LOC, DOC, POC and EOC by 14.73%, 16.5%, 22.5%, 41.5% and 21% in the 0-40 cm soil layer, and by 17%, 14%, 19%, and 30% in the 0-100 cm soil layer. These results suggest that over time, the MBC and MBC-derived C under the fine-sized residue treatment may constitute a significant source of stable SOC through strong physical and chemical bonding to the mineral soil matrix. Conservation management in the North West IGP is important in maintaining soil structure stability and conserving SOC from rapid decomposition with associated organic carbon fractions.

Soil microbial biomass, the active fraction of soil organic matter which plays a central role in the flow of C and N in ecosystems responds rapidly to management practices, and serves as an index of soil fertility. The practices of crop residue retention and tillage reduction provided an increased supply of C and N which was reflected in terms of increased levels of microbial biomass, N-mineralization rate in soil. Residue retention and tillage reduction both increased the proportion of organic C and total N present in soil organic matter as microbial biomass. The no-tillage system showed a trend to accumulate organic carbon near the soil surface layer. Conventional tillage reduced soil organic C stocks and that of its labile fractions both in top and subsoil (20-100 cm). POC reduction was mainly driven by a decrease in fine POC in topsoil, while DOC was mainly reduced in subsoil. Fine POC, LFOC and microbial biomass can be useful early indicators of changes in topsoil organic C. In contrast, LFOC and DOC are useful indicators for subsoil. Reduced proportions of fine POC, LFOC, DOC and microbial biomass to soil organic C reflected the decline in soil organic C quality caused by tillage. The LOC fractions to SOC ratios also decreased, indicating a reduction in C quality as a consequence of tillage and residue management. Reduced LOC fraction stocks in subsoil could partially be explained by the decrease in fine root biomass in subsoil, with consequences for SOC stock.

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# Genotypic differences of soybean (*Glycine max* (L.) Merrill) as a factor of biological intensification of agroecosystems

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**Abstract**— Unfavorable environmental conditions limit the continued yield increases of modern commercial cultivars and hybrids of agricultural plants in the intensive agroecosystems. Therefore, the genotypic differences in resistance/tolerance to biotic and abiotic stresses and the yield of soybean (*Glycine max* (L.) Merrill) are the focus of our long-term studies (2010 – 2018). The soybean breeding lines, collection varieties and commercial cultivars are investigated. The pathogens of viral diseases, namely, the Soybean mosaic virus (SMV) and the Alfalfa mosaic virus (AMV) have been identified. The soybean genotypes having one of such dominant genes as *Rsv1*, *Rsv1t* or *Rsv1y* (locus *Rsv1*) proved to be resistant to local strains of SMV. The genotypes with a relatively high level of the yield and resistance to viral diseases and downy mildew (*Peronospora manshurica* (Naum.) Syd.) are detected. Artificial selection of soybean genotypes for cold tolerance during the seed germination and seedling development period should be carried out taking into account the effect of early planting onto yield components and other plant morphological traits. Soybean yields, as a result of genotype-environment interactions, and the addressed introduction of commercial cultivars into specific agroecosystems are discussed. Selected genotypes can be used in agronomic practice and also as germplasm in breeding of the new high-yielding soybean cultivars with a good adaptability to soil and climatic conditions of Ukraine.

**Keywords**— addressed cultivars, cold tolerance, downy mildew, soybean viruses, yields.

## I. INTRODUCTION

Photosynthesis of agrophytocenoses is a basis of agroecosystems primary bioproductivity. An average radiation-use efficiency (RUE, dry matter produced per unit of intercepted solar radiation) of soybean plants is  $1.02 \text{ g MJ}^{-1}$  [1]. It should be noted, that starting from the moment of assimilates production by photosynthesis and till the accumulation of dry matter in the seeds there are many complex interconnected processes on the molecular, cellular, organismal and population levels which all taken together have a decisive influence on the yield [2, 3, 4, *et al.*].

### 1.1 The importance of soybean genotypic differences in agrophytocenoses

Genotypic differences among soybean plants exist in ability to capture solar radiation by the leaf surface and, as a result, the yield of cultivars may significantly change [5]. Soybean genotypes with reduced chlorophyll content and relatively high photosynthetic activity are promising for the crop sown with an increased plant density [6]. In addition, the ability of agrophytocenosis to intercept and use solar radiation rises in intercropping, compared with monocropping [7]. Therefore, it is possible to increase the use efficiency of soybean agrophytocenoses by selecting genotypes with higher photosynthetic capacity.

Soybean as a legume plant spends up to 20% of photosynthesis products to provide symbiotic nitrogen fixation. In its turn, high nitrogen-fixing activity of root nodules leads to an increase in the photosynthesis intensity and raise in the yield [8]. Genotypic variability in the symbiotic ability of soybean [9] and differences in the nitrogen-fixing activity of rhizobia [10] contribute to the effective combination of high values of the biological nitrogen fixation, photosynthetic capacity and yield of commercial soybean cultivars. For instance, in Brazil, where the average annual soybean yields level is relatively high (over  $3 \text{ t ha}^{-1}$ ), soybean plants receive about 80% of their required nitrogen by symbiosis with nitrogen-fixing bacteria [11, 12]. In this way, the existing genotypic variability of soybean makes it possible to improve naturally nitrogen balance of soil-biotic complex and hence the ecological safety of intensive agroecosystems [13, 11, 14].

### 1.2 Unfavorable environmental factors and soybean yield

Soybean plants are highly sensitive to unfavorable environmental factors, including temperature regime, conditions of moisture supply and diseases. These factors have a negative influence on the yield formation and ultimately lead to significant reduction in its quantitative and qualitative characteristics [15, 16].

In Ukraine, the soybean is represented in agroecosystems by the commercial cultivars with high yield potential and valuable morphological and biological traits. On this basis, they are listed in the State Register of cultivars suitable for introduction and dissemination. However, under conditions of the separate field, farm or group of farms (at the level of the specific agroecosystem) the environmental stress factors can cause yield instability of soybean cultivars. Due to the differences in the level of yield components stability of soybean genotypes [16] it is important to select cultivars that would be able to realize the yield potential under changing environmental conditions.

It's needed to highlight that the breeding programs in the world leading countries at present are increasingly oriented towards creating cultivars and hybrids of agricultural plants characterized not by the maximal yield but the optimally high and stable yield level [17, 18]. Along with the state breeding programs there are private breeding programs in Ukraine. However, it is important to keep the state breeding priorities with new content, since they may not have commercial attractiveness today but are potentially significant in the future.

### 1.3 Research aim

Our long-term main tasks were to search for new approaches, develop and improve the methods of adaptive breeding for optimal combination of the yield, resistance, adaptability and quality in the soybean cultivars. In the breeding process were used the wild-type genotypes (*Glycine soja* Sieb. and Zucc.) which can adequately respond to changing environmental conditions.

## II. MATERIAL AND METHOD

### 2.1 Field experiment description and plant material

Different genotypes of soybean (*Glycine max* (L.) Merrill) including the breeding lines (F8 – F10), collection varieties and commercial cultivars were studied during 2010 – 2018. The breeding lines were created on the basis of hybridization (*G. max* x *G. max*; *G. max* x *G. soja*) and artificial selection for yield and its components, biotic and abiotic resistance /tolerance, early maturity, plant height and other valuable plant traits. Among the collection soybean varieties, 20 varieties with known virus resistance genes were obtained from the US Department of Agriculture (National Plant Germplasm System). Field studies with soybean genotypic diversity were carried out under soil and climatic conditions of Vinnytsia region on the experimental fields of the Vinnytsia National Agrarian University and the Scientific-Production Center "Soybean" of the National Academy of Agrarian Sciences of Ukraine.

In the experimental field soybean seeds were planted manually in 3 rows of plots with a row length of 2.5 m and row spacing of 0.45 m, with four replications. The seeds were planted in soil at a depth of 2 to 4 cm (depending on soil moisture) and seed spacing 5 cm. Two seeds per hole (using a manual planter) were planted in the middle row of each plot. After seedling establishment, the plants were thinned in the middle row with plant spacing 5 cm. Two outer rows were left as borders in addition to the outer 0.25 m at the end of each plot. Phenological observations and biometrics of quantitative traits in the plants were carried out according to methodical recommendations [19]. In the R8 stage (full maturity), 5 plants (in a row) and the plants in a 0.9 m<sup>2</sup> area were collected from the middle row of each plot to evaluate yield and useful economic traits. After measurements, the collected plants were threshed with a threshing machine. Also, the yield components and yield of breeding lines and commercial soybean cultivars were evaluated in another field experiment. The plot consisted of four 10-m sowing rows spaced 0.45 m apart and with seed spacing 3 cm, with three replications. The small farm machinery (seeding machine, rotary hoe and combine harvester) was used in this experiment.

The soybean was sown after winter wheat or barley in the crop rotation. The tillage consisted of the plowing (in autumn) and two or three cultivations (spring seedbed preparation). The fertilizer system included the application of inorganic fertilizers at a ratio of 10 kg N, 20 kg P<sub>2</sub>O<sub>5</sub> and 20 kg K<sub>2</sub>O ha<sup>-1</sup> and seed treatment by rhizobial inoculants. The herbicides were used considering technological recommendations of soybean growing.

### 2.2 Methods for testing biotic and abiotic resistance/tolerance

The genotypic differences of soybean plant resistance to downy mildew and viral diseases were studied in the conditions of natural infection at the R2 stage (full bloom). The disease severity was determined by estimation of 25 to 30 plants of each genotype on a 5-point scale in accordance with the methodical requirements [20]. In virological studies were used methods of the extraction and purification of the local virus isolates (*Soybean mosaic virus* and *Alfalfa mosaic virus*), enzyme-linked immunosorbent assay (ELISA), reverse transcription polymerase chain reaction (RT-PCR), DNA sequencing and electron microscopy [21].

Cold tolerance degree of each genotype in the stage of seed germination was assessed on the basis of seed germinability values at cold (7°C, on the 21st day of germination) and at optimal (23°C, on the 7th day of germination) temperatures (using refrigeration thermostat KBW 720 Binder). Soybean seeds were germinated in the paper, wetted with distilled water in four replications (40 seeds per one). The experiment was conducted in accordance with the ISTA seed germination rules [22]. In the field experiment seedlings were counted on the 28th day after sowing under conditions of early planting (April 1 – 5) and on the 14th day under conditions of optimal planting (late April – early May).

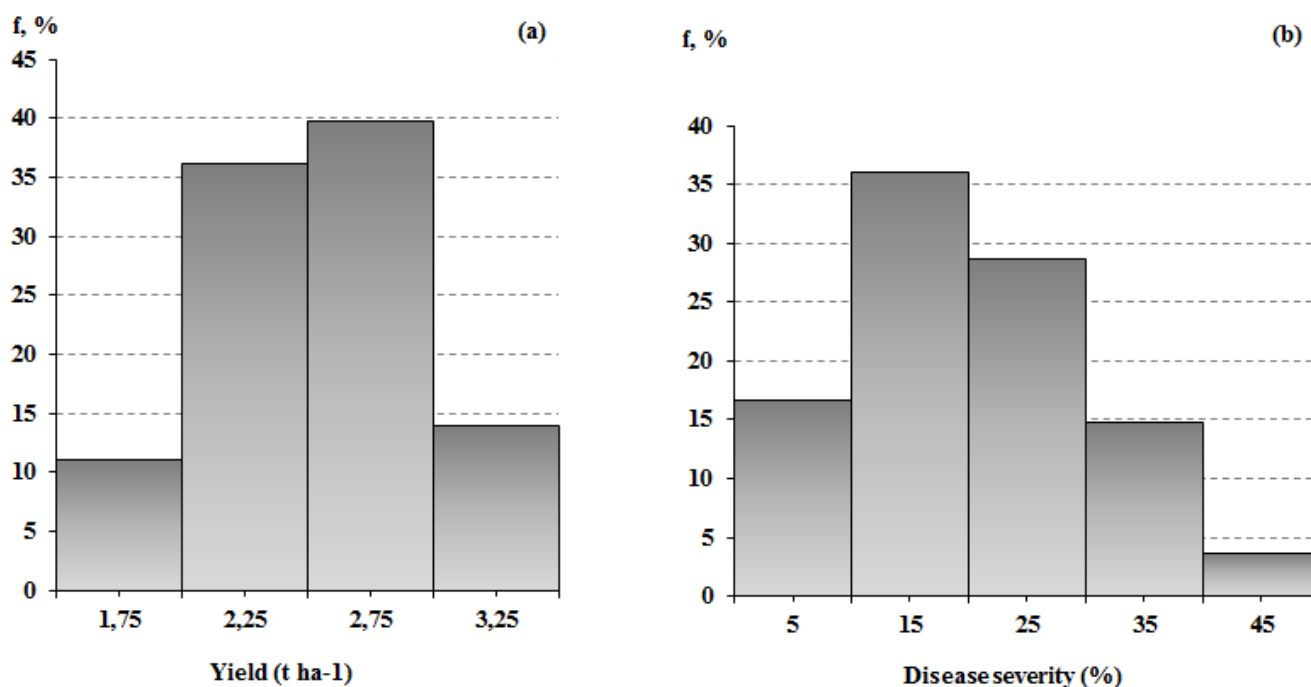
### 2.3 Statistical analysis

Mathematical processing of experimental data was performed by using of Microsoft Excel 2013 and Biostat software packages.

## III. RESULTS AND DISCUSSION

### 3.1 Downy mildew damage of soybean and yield

The frequency analysis of the distribution of soybean breeding lines (on average 100 genotypes per year during 2012 – 2016) according to their yield and plant damage caused by downy mildew infection (*Peronospora manshurica* (Naum.) Syd.) demonstrated differences in phenotypic expression of these characteristics as a result of genotype-environment interactions (Fig. 1). The relatively high level of resistance to downy mildew (disease severity from 0 to 10% on average per plant) was observed in 16.7% of all investigated breeding lines. However, only individual breeding lines (C25712, C80213) differed by the absence of downy mildew symptoms and yielded above 3 t ha<sup>-1</sup>. In most breeding lines with the yield higher than 3 t ha<sup>-1</sup> the disease severity was in the range 1 – 7%. The breeding lines with the disease severity from 30 to 50% were characterized by a low yield (1.5 – 2.0 t ha<sup>-1</sup>) in most cases. At the same time were also found some breeding lines (C36112, C51413) without symptoms of downy mildew lesions but with the yield that did not exceed 2 t ha<sup>-1</sup>.



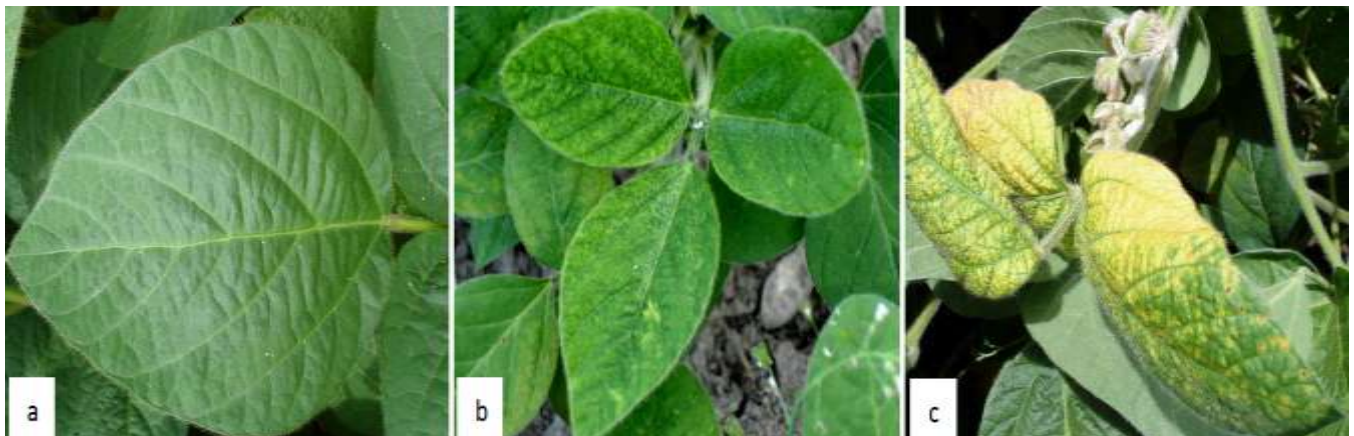
**FIG. 1. Distribution of the soybean breeding lines according to their yield (a) and plant damage caused by downy mildew (b); f – relative frequency; (2012 – 2016)**

In addition, the pathogen resistance and the yield of soybean genotypes were studied on the basis of classical understanding of the "plant-pathogen-environment" interaction system [23, 24]. When environmental factors are more favorable for a plant it can reduce the disease severity and not lead to significant negative pathogen influence onto yield. If environmental factors are more favorable for pathogen the disease severity will increase and it will lead to yield decrease. In case when the vectors of environmental influences onto pathogen and plant coincide, their balanced coexistence would be reasonable. Thus, it is possible to explain the low yield and absence of downy mildew symptoms on the plants of individual soybean breeding lines. Therefore, environmental factors can have a significant modifying effect on both the pathogen resistance and the yield of soybean genotypes, that substantially complicates the artificial selection for combination of these traits.

### 3.2 Viral infection and soybean virus resistance

Significant differentiation of soybean genotypes referring to viral diseases damage severity was found. More than 1000 soybean genotypes were investigated in the field experiments from 2010 to 2018. Such pathogens of viral diseases as *Soybean mosaic virus* (SMV) and *Alfalfa mosaic virus* (AMV) have been identified by the ELISA and RT-PCR methods. As it turned out, the SMV infection was most common among the breeding and collection genotypes of soybean in the conditions of the experimental field of the Vinnytsia National Agrarian University. This infection was identified in more than 70% of the tested genotypes. SMV disease severity on average per plant did not exceed 5% in some collection varieties (Swift, Ajma) only. Whereas the values of disease severity were in the range of 20 – 55% in most other varieties (Aurora, Gamma 85, MON 04, Chernovitska 6, Olima etc.). The AMV infection was identified in less than 10% of soybean genotypes, but the plants infected with this virus could reduce their yield (weight of seeds per plant) by more than 3 times. Besides, the viral infection (detected by the methods of ELISA and electron microscopy) in the plant organism did not always have its phenotypic expression (the infection was in the latent state).

The collection varieties of soybean with known SMV resistance genes (loci Rsv1, Rsv3, Rsv4) [25] were investigated under conditions of natural infection in relation to their ability to resist local SMV strains. Among these varieties, both resistant (plants without symptoms of viral infection) and susceptible (plants with symptoms of systemic viral infection, including leaf necrosis and stem-tip necrosis) genotypes were detected (Fig. 2). The soybean genotypes (York, Ogden, Suzumar, PI96983, Hourei, PI 398476, VIR 2980, PI 486355 varieties) which have one of the dominant genes Rsv1, Rsv1t or Rsv1y (each alone or in combination with genes Rsv3 and Rsv4) were resistant to local SMV strains. Severally, Rsv3 and Rsv4 were ineffective genetic determinants of resistance to local SMV strains. Although according to the studies [26], the Rsv4 gene can provide resistance to a wide range of SMV strains. Ukrainian isolate of SMV named UA1Gr was identified as belonging to the G4 strain group [27].



**FIG. 2: Soybean leaf without symptoms of viral infection (a); Soybean leaves with systemic mosaic symptoms (b) and stem-tip necrosis (c)**

The *Rsv1-f/-r* gene-specific to the *Rsv1* locus molecular marker [28] and the Satt542 and Satt634 microsatellite markers linked with the locus *Rsv4* [26] were used for the genotypic identification of commercial soybean cultivars for resistance to SMV infection. It was determined that the high-yielding cultivars Vinnychanka and Gorlytsia (the cultivars created by the adaptive breeding methods) have the effective genetic determinants of resistance to SMV infection. These cultivars can be used in agronomic practice and also as germplasm in the selective breeding of new soybean cultivars with virus resistance and good adaptability to soil and climatic conditions of Ukraine.

### 3.3 Soybean genotypic differences in low-temperature sensitivity

Formation of high-yielding soybean agrophytocenoses largely depends on the abiotic factors. In particular, soybean requirements to warmth appear in the stages of seed germination and seedling development. Soybean seedlings appear 5 – 7 days after sowing under favorable temperature conditions (20 – 25°C). But a decrease in temperature to 7 – 10°C slows the seed germination and delays the emergence of seedlings for up to 25 days or more. Therefore, an increase in cold tolerance of

commercial soybean cultivars during the seed germination and seedling development period would contribute to the utilization of spring soil moisture by plants more efficiently. That would lead to the activation of growth and photosynthetic processes, and consequently to the increase of quantitative and qualitative characteristics of the yield.

Significant differences in cold tolerance at the seed germination and seedling emergence period were found among the soybean breeding lines. The genotypes (C88812, C11512, C84912, C23112) with comparatively high values of seed germinability (above 90%) under low-temperature conditions were identified in the laboratory and field experiments (Table 1). The genotypes with the inferior ability of seeds to germinate under conditions of low temperatures were detected too. Seed germinability of the investigated genotypes was above 90% under the optimal temperature regime (23°C).

**TABLE 1.**  
**COLD TOLERANCE ESTIMATION OF THE SOYBEAN BREEDING LINES AT THE SEED GERMINATION AND SEEDLING EMERGENCE PERIOD (ON AVERAGE DURING 2014 – 2017)**

Breeding line	Seed germinability (mean ± SE), %		
	laboratory conditions		early planting under field conditions
	23°C	7°C	
C88812	96.9	94.29± 1,96	91.88± 2,16
C11512	94.4	93.57 ±2,07	91.25± 2,23
C89712-2	92.5	92.86 ±2,18	83.13± 2,96
C84912	96.3	92.14 ±2,27	90.63 ±2,30
C23112	91.9	91.43 ±2,37	93.12± 2,01
C39811	96.3	86.43± 2,89	73.38 ±3,49
C16112	93.8	81.42± 3,29	91.25 ±2,23
C83012	94.4	70.71 ±3,85	68.13± 3,68
C84212	95.0	72.14 ±3,79	72.50 ±3,53
C23911	93.1	62.86 ±4,08	66.88± 3,72
Negrutsa*	96.3	92.86 ±2,18	90.63± 2,30

$t_{01} = 2.63$

*SE* – standard error;  $t_{01}$  – value of Student's *t*-test at 99% confidence level; \* – collection variety, cold-tolerance check indicator

Early planting contributed to the differentiation of breeding lines with respect to the yield and other important agronomic traits. In particular, the plant height at maturation (on average during 2014 – 2017) in more than 80% of breeding lines was decreased within 10-25 cm depending on the genotype under early planting (April 1 – 5) compared to optimal planting (late April – early May). A significant decrease in yield (more than 0.5 t ha<sup>-1</sup> on average during 2014 – 2017) was observed in most breeding lines under early planting conditions compared to optimal planting. The genotypes (C84912, C23112) with the yield of more than 3 t ha<sup>-1</sup> by planting early and the yield of less than 2.5 t ha<sup>-1</sup> under optimal planting were also found. In addition, the genotypes (C88812, C11512) whose yields remained relatively high (2.9 – 3.4 t ha<sup>-1</sup>) at both planting dates were detected.

Previous studies of the genetic control of soybean cold tolerance have shown the relative independence of genetic factors which define the cold tolerance in the stages of seed germination, seedling development, and bloom and pod formation [29]. Therefore, the evaluation and selection of soybean genotypes for cold tolerance should be conducted differentially considering the ontogenesis stage. Besides, the artificial selection of soybean genotypes for cold tolerance during the seed germination and seedling development period should be carried out taking into account the effect of early planting onto yields and other important agronomic traits.

### 3.4 Yield of soybean in Ukraine

The analysis of soybean world production indicators (Table 2) shows that more than 55% of the sown areas and more than 65% of the grain production of soybean belongs to the USA and Brazil. The yield potential of soybean cultivars is effectively realized (3.3 – 3.5 t ha<sup>-1</sup>) in these countries. Therefore, the average yield of this crop in the world is relatively high and is over 2.7 t ha<sup>-1</sup>.



In Ukraine, the average soybean yield during 2016 – 2018 varied from 2.0 to 2.6 t ha<sup>-1</sup>. It is significantly lower than the average yield achieved by the leading soybean-producing countries in the world (tab. 2). Though, it should be noted that there is a sufficient potential of cultivars in Ukraine to increase the average yield of soybean. Currently, the State Register includes more than 200 high-yielding commercial soybean cultivars of the domestic and foreign breeding which are able to achieve the yield higher than 3 t ha<sup>-1</sup> in favorable growing conditions. However, the unfavorable environmental factors (pathogens, low and high temperatures, moisture shortages and others) cause the significant differences in yields among cultivars depending on their resistance/tolerance and adaptability to environmental factors. The changes in yields among cultivars under unfavorable environmental conditions have a negative effect on the average soybean yield. Furthermore, the cultivation factors contributing to the high yield realization of cultivars in optimal conditions can be completely ineffective under stress [12].

**TABLE 2.**  
**THE MAIN INDICATORS OF MODERN WORLD SOYBEAN PRODUCTION (USDA, DECEMBER 2018) [30]**

Year	World	USA	Brazil	Ukraine
<i>Area (million hectares)</i>				
2016	119.76	33.47	33.90	1.86
2017	124.69	36.23	35.15	1.98
2018	128.31	35.75	36.20	1.70
<i>Production (million metric tons)</i>				
2016	349.30	116.92	114.60	4.29
2017	339.47	120.04	120.30	3.89
2018	369.20	125.18	125.18	4.40
<i>Yield (t ha<sup>-1</sup>)</i>				
2016	2.92	3.49	3.38	2.31
2017	2.72	3.31	3.42	1.97
2018	2.88	3.50	3.37	2.59

The addressed introduction of cultivars into specific agroecosystems is one of the effective ways to solve the problem of the average soybean yield raising and stabilization. This is carried out on the basis of cultivars with high adaptability to the soil-climatic and technogenic conditions of specific agroecosystems. Cultivars with high adaptability to specific agroecosystems might be called the "addressed cultivars". The addressed introduction of commercial soybean cultivars is important for Ukrainian farmers who aim to stabilize the average yield level of this crop above 3 t ha<sup>-1</sup> [31].

#### IV. CONCLUSION

Soybean genotypic differences in resistance/tolerance to biotic and abiotic stresses, in yield components and other valuable morphological traits constitute the biological basis for a formation of the effective agrophytocenoses. It is necessary to evaluate breeding lines and commercial cultivars under conditions of specific agroecosystems where their addressed introduction is expected. Selected soybean genotypes (the best recombinant inbred lines and cultivars) can be used in agronomic practice and also as germplasm in breeding of the new high-yielding cultivars with good adaptability to soil and climatic conditions of Ukraine.

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# The Cation Exchange Capacity, pH of Soil in Mwogo Marshland, and the Rice Plantation in Huye District -Rwanda

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**Abstract**— Agriculture is a major component of Rwanda's national economy. In 2017, agriculture contributed 33% to the country's GDP. About 66.46 % of population, of which 50.9 % are women, depends either directly or indirectly on agriculture for living. The average arable surface area available is about 0.60 ha per household use. This causes overexploitation of available land which is often accompanied by agricultural malpractices with disastrous consequences on land resources and on environment in general. Given the limited availability of arable land for agriculture and the constantly growing food requirements of the population, ensuring food security poses a major challenge. This present study aimed at investigating the cation exchange capacity and pH of soil of Mwogo Marshland in order to resolving the problem of soil fertility of Mwogo marshland by looking the method for increasing its fertility and then the problem of low rice production. By using soil Auger, samples were taken randomly in the field where each sample of soil was used in laboratory to determine both pH and Cation Exchange Capacity, in each blocks namely Block du Nord and Block du Sud. During this study the laboratory results and laboratory analysis has shown that marshland soil is very acidity with pH<sub>hcl</sub> is 4.37, Ph water with a weak cation exchange capacity. These findings support the previous studies showing that the soils with those properties need particular management; like liming, addition of organic matter, and so on, in order to adjust its chemical properties.

**Keywords**— Soil, chemical properties, rice production.

## I. INTRODUCTION

Soil property influencing soil structure stability, nutrient availability, soil pH and the soil's reaction to fertilizers and other ameliorants (Hazleton and Murphy 2007). The Cation Exchange capacity of soils varies according the type of soil, soil pH and amount of organic matter, for example the pure sand soil is contains low cation exchange capacity, less than 2 meq/100 g. The research of Mckinze stated that soils with large quantities of negative charge are more fertile because they retain more cations (McKenzie *et al.* 2004) however; productive crops and pastures can be grown on low cation exchange capacity soils. The main ions associated with cation exchange capacity in soils are the exchangeable cations calcium (Ca<sup>2+</sup>), magnesium (Mg<sup>2+</sup>), sodium (Na<sup>+</sup>) and potassium (K<sup>+</sup>) (Rayment and Higginson 1992). However, as soils become more acidic these cations are replaced by H<sup>+</sup>, Al<sup>3+</sup> and Mn<sup>2+</sup>, which affect soil fertility and some plants do not able to grow in that situation (McKenzie *et al.* 2004). As a consequence, Brady (2012) argued that acidic soils, range of hydrologic and climatic condition are affecting growth and production of rice (Brady, 2012).

In small and very densely populated country Rwanda, the average arable surface area available is about 0.60 ha per household lead people to overexploit land activities, convert pastures and woodlots into cropland and cultivate in malpractice which fragmented, fragile, steep-sloping of areas and cause also soil infertility ( Imerzoukene and Van Ranst, 2001). Other characteristics of the subsistence agriculture in Rwanda are the lack of individual and regional specialization, a weak integration between agriculture and the economic markets, and an important dependence on the climatic conditions which resulting a serious decrease of the physical and chemical soil fertility and affect crop yields (Imerzoukene and Van Ranst, 2001).

Rice plantation has been adapted as major crop need empowerment in Rwanda in order to cop that challenge of food insecurity. In the past 10 years, the total rice production has increased by 6-fold from 11,949 tons in 2000 to 72,000 tons in 2009. Rwanda annually imports Tanzanian, Indian and Pakistan long grain rice: 57,229 MT (2012) while Rwanda produced only short grain rice (Kigori): 81,908 MT (2012) which is very low compare to the needs of population and lead the rice consumption be outstripped to the local production.

In the same case, the rice productivity of Mwogo marshland is critical due to the soil nutrient depletion which is directly linked to food insecurity of country and local community. Many factors are associated such as nitrogen, phosphorus, Insufficient mechanization of farming operations, therefore this study was focused on effect analysis of cation exchange

capacity and soil pH on soil fertility of Mwogo marshland thereby the soil fertility continues to decline and this declining is showed by low production of rice. This study aims to end up by resolving the problem of soil fertility of Mwogo marshland by looking the method for increasing its fertility. The specific objectives of this study are to: Assess soil PH and cation exchange capacity of mwogo marshland, determine the effect of CEC and soil Ph of Mwogo marshland, to determine strategic way of increasing rice productivity in Mwogo marshland to measure the rate of cations exchange capacity in Mwogo marshland.

## II. METHODOLOGY

The marshland of Mwogo is located in southern province, Huye district, on an altitude comprise between 1572 and 1715 m above the sea level. The latitude on which mwogo marshland is located on is at 2°36' south of the equator; 29° 49'51'' and 29° 44' East of the Greenwich meridian. Mwogo marshland is about 500ha and is located in Huye district and passes through Rwaniro, Kigoma and Simbi sectors all around the river called Mwogo. It is located in south-east shelf of the tropical agro-climate zone. Soil of Mwogo marshland derives from schisto-quartzites mica material formed at the basin side; the soils are generally clayed, deep, and red with an advanced alteration (Mbonigaba, 2002) .

A simple random sampling was used to select two blocs, where one bloc were taken in northern part of mwogo and another bloc in southern part, three different rice paddy fields( plots) were taken at each bloc and one sample were taken in each plots of the bloc at depth of 20-50 cm below surface with an auger. The collected soil samples were dried for 7days at the normal laboratory temperature and sieved at 2mm for testing soil PH by using deionised water (distilled water) and KCl. The 25ml of the soil solution was added 10ml of NaOH and put into the distillatory for heating and evaporation. Then after the cooled solution felt down in the beaker containing 5ml of Boric acid and wait 450seconds. The process coloration was changed from reddish to the blue color for titration.

CEC was determined by Metson method which consists of extraction with ammonium acetate 1N at pH 7, followed with distillation and titration by hydrochloric acid 0.1N. In fact, by taking 25ml of the soil solution; and add 10ml of NaOH then put them in the upper part of the distillatory, Below there was a tube containing water. The water was heated until to evaporate. The water vapor heated the mixed solution of the soil sample and NaOH until to evaporate in return. The vapor from the solution was cooled down by the water from the tap circulating in the distillator and removed to fall in lavabo. The cooled solution felt down in the beaker containing 5ml of Boric acid. We stopped the process after 450seconds and then during the process the reddish color changed to become blue.

Thereafter, the solution settled down in three beakers was titled by HCl 0.1N where the following volume of HCL 0.1N were used to titrate solutions in beakers:

Blank : 0.25ml of HCL 0.1N

Soil sample from Block I : 10.7ml of HCL 0.1N

Soil sample from Block II: 2.6ml of HCL 0.1N

### 2.1 Titration

- ✓ Titrate solutions in beakers by HCL 0.1N
- ✓ Wait so that the solution color changes to red.
- ✓ Note the volume of HCL 0.1N used.

### 2.2 Calculations

Correction factor =4

$$\text{CEC (meq/100g of soil)} = \text{titrated volume of sample -- titrated volume of temoin} *4$$

## III. RESULTS

The cation exchange capacity was different from different location of marshland, in northern part of Mwogo, the cation exchange capacity falls in the range of 9.6 and 10.42 within the soil depth of 20-50 cm. as it is shown in table 1 which means that northern part of Mwogo is highly sand soil, Nitrogen and potassium leaching more likely, less lime required to correct a

given PH, physical ramifications of a soil with a high sand content, and finally low water holding capacity. In the southern part the cation exchange capacity falls in the range of 7.03-9.34 within the soil profile of 20-50 cm as it is indicated in table 2 which means that the soil of southern part of mwogo is moderated clay.

**TABLE 1**  
**CATION EXCHANGE CAPACITY OF NORTHERN MWOGO MARSHLAND**

SITE	Plots number	Soil depth (cm)	CEC(cmol/kg of the soil)
NM	Plot no 1	20-50	9.6
	Plot no 2	20-50	9.99
	Plot no 3	20-50	10.42

*Legends: NM: Northern Mwogo*

**TABLE 2**  
**CATION EXCHANGE CAPACITY OF SOUTHERN MWOGO MARSHLAND.**

SITE	Plots number	Soil depth (cm)	Cec cmol/kg of the soil
SM	Plot no 1	20-50	9.34
	Plot no 2	20-50	9.20
	Plot no 3	20-50	7.03

The table 3 results showed that CEC of Mwogo marshland has 9.198 which is weak.

This is due to high sand and low clay content, low organic matter due to washing away of the organic matter by a flow of water, low organic matter content, low water-holding capacity which makes bases to leach into the soil, low soil pH and enable to resist on changes in pH or other chemical changes due to high potential of leaching of base cations in the deeper layers of the soil. This weak cation exchange capacity affect the soil properties and the rice crop in faster decrease of soil pH, need to be limed more often, low nutrients available for plant, high amount of essential elements leached into the soil.

**TABLE 3**  
**AVERAGE CATIONS EXCHANGE CAPACITY OF MWOGO MARSHLAND**

Blocks	Plots	CEC NM	CEC SM	Aver. CEC
B1	plot1	9.6	9.34	9.47
B1	plot2	9.99	9.2	9.595
B1	plot3	10.42	7.03	8.725
B2	plot1	9.7	9.09	9.395
B2	plot2	10	8.91	9.455
B2	plot3	10.1	7	8.55
AVERAGE		9.96833333	8.42833333	9.198

In the part of northern part of mwogo marshland, the soil profile was measured with two methods; pH water and pH<sub>KCl</sub> and the results are the following: pH<sub>KCl</sub> falls in the range of 4.30-4.61 which means that the pH of this soil is very acid and pH water falls in the range of 5.30-5.62 which means this soil is fairly acid as it shown in table 4.

**TABLE 4**  
**RESULTS OF PH OF NORTHERN MWOGO MARSHLAND SOILS**

Sites	Plots number	Soil depth (cm)	pH <sub>KCl</sub>	pH <sub>water</sub>	△ pH
NM	Plot no 1	20-50	4.30	5.30	-1.00
	Plot no 2	20-50	4.42	5.55	-1.13
	Plot no 3	20-50	4.61	5.62	-1.01

*Legends; NM: Northern mwogo.*

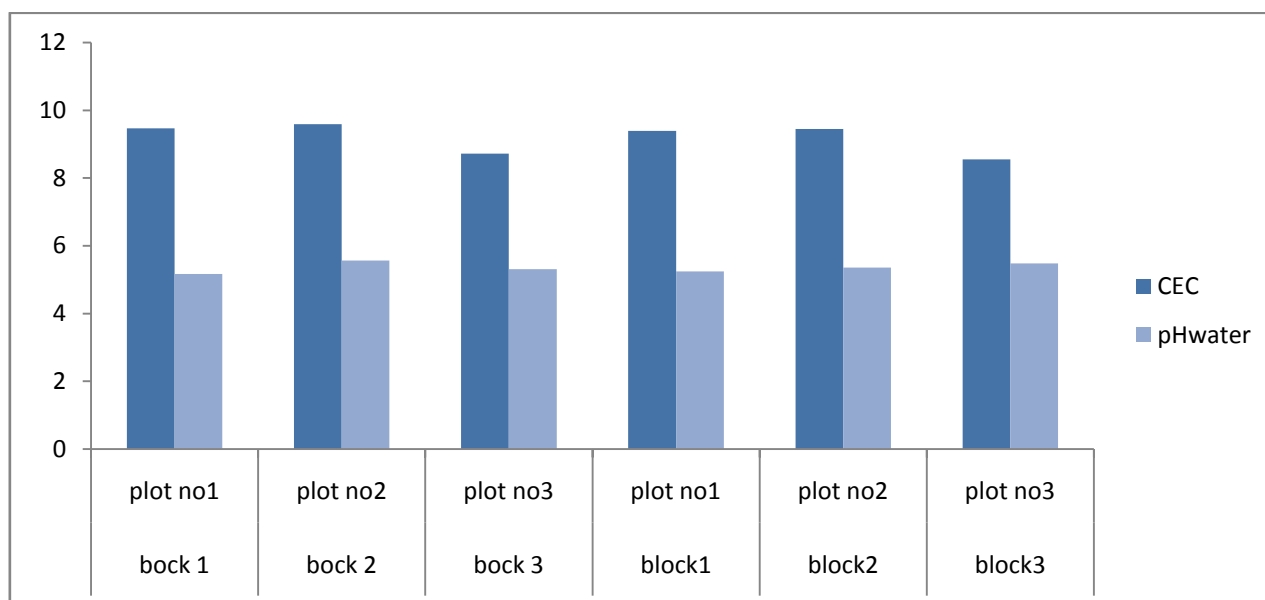
In the part of southern part of Mwogo marshland, the soil pH water and pH<sub>cl</sub> and the results are in the range of 4.06-4.13 which means that the pH of this soil is very acid and pH water falls in the range of 5.00-5.60 which means this soil is fairly acid as it is indicated in bellow table five.

**TABLE 5**  
**RESULTS OF pH OF SOUTHERN MWOGO MARSHLAND SOILS**

Sites	Plots number	Soil depth (cm)	pH <sub>cl</sub>	pH <sub>water</sub>	△ pH
SM	Plot no 1	20-50	4.13	5.04	-0.91
	Plot no 2	20-50	4.18	5.60	-1.42
	Plot no 3	20-50	4.06	5.00	-0.94

*Legends: SM: southern mwogo,*

The bellow bar chart (figure 2) shows that in Mwogo marshland the cations exchange capacity of the soil increases with the increase of soil Ph as it is indicated by figure 1.



**FIGURE 1: Relationship between CEC pH of the soil.**

#### IV. DISCUSSION

In northern part of mwogo, the cation exchange capacity falls in the range of 9.6 and 10.42 within the soil depth of 20-50 cm. this means that northern part of mwogo is characterized by:

High sand content, Nitrogen and potassium leaching more likely, less lime required to correct a given PH, physical ramifications of a soil with a high sand content, and finally low water holding capacity. In the southern part of mwogo marshland, the cation exchange capacity falls in the range of 7.03-9.34 within the soil profile of 20-50 cm.

Average cation exchange capacity of mwogo marshland is 9.19 which means that the soil have weak cation exchange capacity. This is due to high sand and low clay content, low organic matter due to washing away of the organic matter by a flow of water, low organic matter content, low water-holding capacity which makes bases to leach into the soil, low soil pH and enable to resist on changes in pH or other chemical changes due to high potential of leaching of base cations in the deeper layers of the soil.

This weak cation exchange capacity affects the soil properties and the rice crop production decrease of soil pH, need to be limed more often, low nutrients available for plant, high amount of essential elements leached into the soil. The pH<sub>water</sub> of the soil of mwogo marshland decreases from the northern mwogo in plot n<sup>o</sup>3(5.62) to southern mwogo in plot number n<sup>o</sup>

3(5.00), these are more or less drained profile, they are located not far away from the hillsides, and contain more acidifying cations. But the cations exchange capacity is affected positively; it increases from 7.03 Cmol (+)/kg of the soil in southern mwogo plot number 3 to 10.42 Cmol (+)/kg of the soil in northern mwogo plot number 3.

This is explained by the fact that there was more organic input from old plants decomposing under the upper layer of the soil, at organic horizon level. The organic matter has capacity to hold the base cations. As it is visible, cations exchange capacity increases and decreases with the Ph. PhKcl decreases from 4.61 to 4.06. The average pH<sub>water</sub> of mwogo marshland is 5.35 and the average pH<sub>kcl</sub> of this marshland is 4.3. This affects negatively the exchangeable bases decreasing from 10.42 to 7.03. The phenomenon is explained by the fact that there is a transport of colluviums and alluviums from the hillsides to the valley by the erosion. Recall that this region is one of regions of southern province which register more rainfall.

The PH of mwogo marshland is moderately acidic, the difference between pH<sub>kcl</sub> and pH<sub>water</sub>, shows that these soils are capable to fix some more cations because they have some negative charges from small amount of organic matter from dead plant and animals. The average soil pH<sub>kcl</sub> of mwogo marshland falls on 4.37 and the average pH<sub>water</sub> of this marshland is 5.35. This means that mwogo marshland is fairly acidic according to Mutwewingabo and Rutunga, 1987, where my research findings coincide with findings of other researchers like Rayment and Higginson 1992, Mutwewingabo and Rutunga, 1987 and McKenzie *et al.* 2004.

The factors of this acidification are the following: accumulation of organic matter which are from hills side due to erosion and high rain fall registered with this region, breakdown of primary minerals (high weathering) and leaching of soluble constituents due to low holding capacity as shown by its soil cation exchange capacity.

Acidity of this marshland affect rice crop through low nutrients absorption, low availability of nutrients, salt effect.

## V. CONCLUSION AND RECOMMENDATION

The study showed that soil of Mwogo marshland is acidic soil and its CEC is weak means that mwogo marshland require particular measures for increasing its productivity. Acidic conditions and its cation exchange capacity need to be ameliorated because it is not suitable for better growth of rice. Therefore, we recommend reducing soil acidity, efficient use fertilizer, improving decomposition of crop residues for Ph case while for Cation exchange capacity improvement of CEC in weathered soils by adding lime and raising the Ph is needed. The use organic matter is the most effective way of improving the CEC in this marshland.

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# Forage plants in Daloa city livestock market: specific diversity, market practices and economic land

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**Abstract**— *The sale of forage is little known to the majority of people in Côte d'Ivoire. The target of the study is to identify the forage species marketed in the livestock markets of Daloa and to estimate the financial profitability of this activity in the socio-economic life of the actors of the sector. Semi-structured surveys were conducted from September to December 2018 among 45 vendors in the forage marketing chain. Nine forage species divided into five genera and four families were identified. The study showed that these plants come from the non-agglomerated areas of the city, fallows and old plantations. The main species are f forage Moraceae, especially *Ficus exasperata*, highly sought after by customers. The average selling price of a forage species boot is around 100 to 150 FCFA. The estimated average daily financial income per player is FCFA 750 and varies between 18,750 to FCFA 37,500 per month for a monthly average of FCFA 26,125 and oscillate between 225,000 to FCFA 450,000 for an annual average of 313,500 FCFA. However, although the harvesting and sale of forage trees is a pathway generating substantial income, it is a source of degradation of plant formations already overexploited in Côte d'Ivoire.*

**Keywords**— *Forage plants, livestock markets, financial income, actors, Daloa, Côte d'Ivoire.*

## I. INTRODUCTION

In Côte d'Ivoire, the economy is essentially based on agriculture, which employs more than 2/3 of the active population, and contributes 34 percent of total GDP and export income for 66 percent [1].

Livestock farming, which for a long time remained the poor relation of Ivorian agriculture, is enjoying renewed interest. Despite the difficulties encountered, the animal resources sector contributes about 4.5 percent to agricultural GDP and 2 percent of total GDP, and employs around 360,000 people [2]. Livestock farming is practiced throughout the Ivorian territory and plays an important role in the national economy.

Today, the coverage rate of national meat and offal requirements is 26.69 percent and will increase to 61.62 percent, a challenge that remains to be achieved for Côte d'Ivoire by 2020 [1]. In this perspective, in many Ivorian cities, there is no less important livestock markets, where urban and traditional livestock farming activities, whether for-profit or not, are carried out by the population [3].

Activities in these livestock markets make an essential contribution to the domestic economy of cities [4-5]. Also, the feeding of domestic ruminants remains a challenge to be mainly forage spontaneous plants from the islets of natural formations and fallow land in the peripheral areas of cities in the tropical regions of Africa [6].

According to [7], the grazing of spontaneous species from these natural formations is generally the only way to feed cattle, sheep and goats in urban areas. Indeed, in these African cities, and in particular in Daloa, for livestock feed requirements, livestock owners are resorting more and more to forage crops. They are collected very often in areas not yet agglomerated in the city, fallows or lowlands and sold on livestock markets of small ruminants. The main issue is the financial profitability of commercial forage activity in urban Daloa.

This study is a contribution to the collection of useful information on the collection and sale of forage crops in Daloa livestock markets. The general target of this study is to identify the forage species marketed on the Daloa cattle markets and to estimate the financial profitability of this activity in the socio-economic life of the actors of the sector.



Specifically, this will involve: (1) investigating forage plants marketed in livestock markets in the City of Daloa; (2) identifying forage species sold; (3) knowing their areas of provenance and (4) estimating the financial profitability of this activity of collection and sale of forage plants in the socio-economic life of the actors of the sector.

## II. MATERIAL AND METHODS

### 2.1 Study sites

The study was conducted in Daloa, a city in west-central Côte d'Ivoire, in the Haut-Sassandra region with geographic coordinates of 6°53 N latitude and 6°27 W longitude (Figure 1).

The climate of the study area belonging to the equatorial domain is of tropical type with two rainy seasons (april-july) and (september-november), two dry seasons (december-march) and (july-september). The rainfall varies 1300 mm between the driest month and the wettest month of 1800 mm.

The choice of this study area is justified by the fact that Daloa, because of its agricultural potential, is a cosmopolitan city where many ethnic groups coexist, some of which are traditionally breeders in which the activity of urban breeding is practiced. In addition, the transport of forage plants by means of various means is used on the urban roads in order to supply livestock markets for small ruminants and slaughterhouses. Also, in these markets, the marketing of forage plants is a daily practice that occupies more and more a certain part of the population.

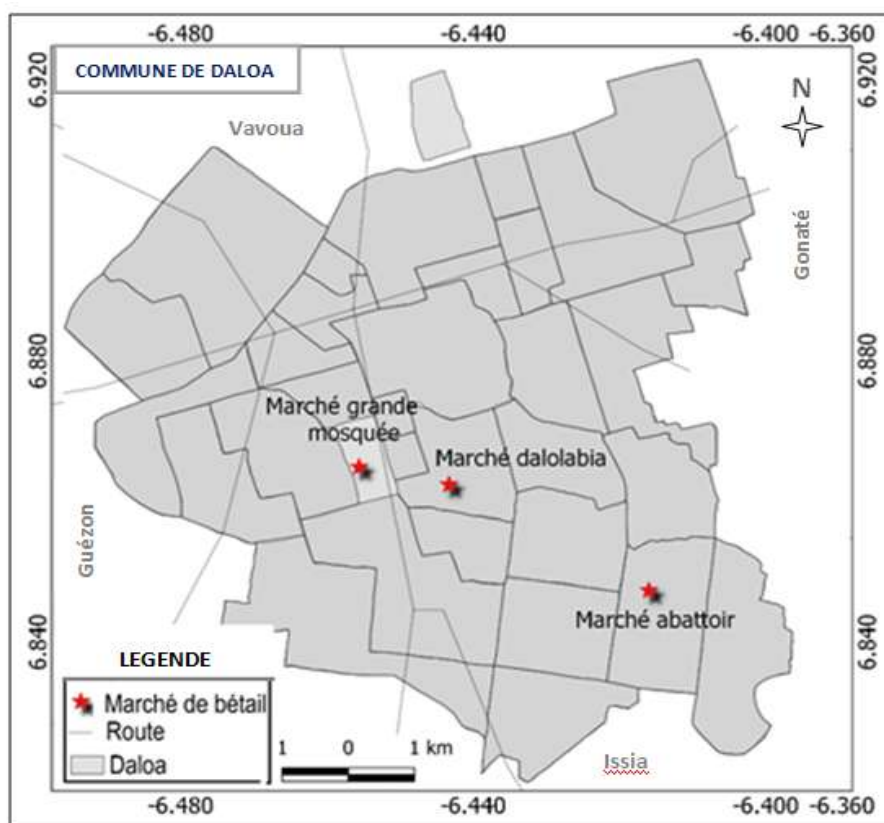


FIGURE 1: Presentation of survey sites in the city of Daloa

### 2.2 Surveys and identification of forage plant species

The investigations were conducted from september to december 2018 on three cattle markets in the city of Daloa with geographical coordinates: big mosque market (06°87, 203'N and 006°44, 341'W), slaughterhouse market (06°87, 411'N and 006°44, 853'W) and dololabia market (06°87, 309 and 006°44, 418'W) on which forage plants are sold for feeding to ruminants (Figure 1).

To conduct this study well, an exploratory survey was carried out in the city in september 2018 and allowed to identify 98 individuals, actors in the chain of sale of forage plants. These people served as a sampling base. From this basis, a random

draw was used to retain forty-five (45) forage vendors regardless of gender, age and ethnicity. Surveys of these forage vendors were conducted using semi-structured interviews.

The interview was conducted in French sometimes, in local languages with the help of interpreters, sometimes followed by sampling of commercialized forage plants.

The estimate of sales by bundles and not in kilograms of forage plants was made, first on the basis of the daily sale, then on the basis of the number of days of sale in the week, the value of sales per week, then by month and finally by year.

The sections of the survey sheet cover the socio-economic characteristics of the sellers of forage plants, their areas of provenance, the names of the forage species sold, the selling price of forage species and the profitability of the marketing activities of forage plants have been mentioned. The obtained plant samples were identified at the National Floristic Center (CNF) of Abidjan.

### III. RESULTS

#### 3.1 Socio-demographic characteristics of the sellers of the forage value chain

For this study, forty-five (45) actors in the forage chain were interviewed in three markets in the city of Daloa (Figure 2). The majority of the actors interviewed are men (100 percent). The age of the respondents varies between 23 and 52 years with a majority of over 46 years (Table 1).

The results of the surveys conducted on the marketing sites for woody forage species made it possible to identify the main strategic players in the fodder trade chain. These are simple transporters, simple collectors, collectors/sellers, wholesalers and customers (livestock traders). Of the 45 actors interviewed, 40 percent were men over 44 (Figure 3) and 46, 66 percent of young people over 25 years old.

For the forage traders surveyed, the sale of forage species is a permanent activity for 46.67 percent of salesmen with more than four (4) years of experience. Also, the sale of these forages is an activity carried out by several ethnolinguistic groups with a sub-regional predominance of two majority ethnic groups: Peuhl (31.11 percent of the ethnic groups) from Mali and 15.56 percent by the Mossis (Burkina Faso).



FIGURE 2: Slaughterhouse market and forage sale site in Daloa

**TABLE 1**  
**SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE INTERVIEWED OPERATORS**

Characteristics		Number of individuals	Frequencies
Genus	Man	45	100
	Women	0	0
Age	≤ 25 years	3	6,67
	> 25 to ≤ 35 years	21	46,66
	> 35 to ≤ 45 years	18	40
	> 45 years	3	6,67
Year of experience	≤ 1 years	20	44,44
	> 1 to ≤ 5 years	21	46,67
	> 5 years	4	8,89
Level of study	Illiterate	36	80
	Primary	2	4,44
	Secondary	7	15,56
Main ethnic groups	Mossi	7	15,56
	Peul	14	31,11
Nationality	Burkinabe	13	28,89
	Ivorian	8	17,78
	Malian	24	53,33
Distribution of actors interviewed by market	Slaughterhouse market	11	24,44
	Big mosque market	25	55,56
	Dalolabia market	9	20
Distribution of actors encountered	Carriers	3	6,66
	Collectors / sellers	18	40
	Vendors wholesalers	8	17,77
	Customers	16	35,56



**FIGURE 3: A forage trader interviewed in the livestock market in Daloa**

### 3.2 Diversity of forage plants marketed

A total of nine (9) commercial forage species were identified (Table 2) in three (3) livestock markets in Daloa city (big mosque market, slaughterhouse market and Dalolabia market). These forage species are divided into five (5) genera and four (4) families. The most represented family is the family of Moraceae with five (5) species, either 55.56 percent (Table 2). Of

these nine (9) woody forage species, seven (7) are shrubs, either 77.78 percent, and two (2) are trees (22.22 percent). *Ficus exasperata* is the most sought-after and best-selling forage species on the three (3) markets.

**TABLE 2**  
**FORAGE SPECIES IDENTIFIED ON THE DALOA LIVESTOCK MARKETS**

Scientific name	Family	Common names	Biological type	Interest
<i>Albizia zygia</i> (DC.) J.F Macbr	Fabaceae	Sili	Tr	**
<i>Baphia nitida</i> Lodd.	Fabaceae	Bènbe	Sh	*
<i>Ficus capensis</i> Thunb	Moraceae	Grattoir	Sh	**
<i>Ficus exasperata</i> Vahl	Moraceae	Grattoir, folokâ	Sh	***
<i>Ficus sur</i> Forssk.	Moraceae	Grattoir	Sh	**
<i>Ficus umbellata</i> Vahl	Moraceae	Popo	Sh	**
<i>Ficus vallis-choudae</i> Delile	Moraceae	Grattoir	Sh	**
<i>Gmelina arborea</i> Roxb. Ex Sm	Verbenaceae	Nanuyi, gopo	Tr	*
<i>Griffonia simplicifolia</i> (DC.) Benth.	Caesalpiniaceae	Npèku,	Tr	**

*Tr* - Tree; *Sh* - shrub; \* - Lowly sought species; \*\* - Moderately sought species; \*\*\*- Highly sought after species

### 3.3 Areas of origin of fodder marketed in the city

In the three livestock markets, the main periurban forage supply routes identified are of the order of three (3). These are the Gonaté-Daloa, Vavoua-Daloa and Issia-Daloa road. The Gonaté-Daloa axis alone provides 68 percent of the supply of the three markets for spontaneous forage species. Among the nine (9) fodder species marketed, *Ficus exasperata* is the forage species highly sought after by all stakeholders in the value chain of forage species interviewed.

Survey results show that the marketed forage comes mainly from fallow land, small patches of natural formations, old plantations and fields along the major roads of the periurban areas of Daloa. These harvesting areas are generally located on a 15 km radius around the city of Daloa over the three (3) identified supply routes. Spontaneous forage plants are sold in bundles fresh and not in kg.

### 3.4 Economic impact of the forage trade

The marketing of fodder plants is an economic activity that generates a variable daily profit depending on the actors of the trade in forage species. The transport of forage trees to livestock markets is carried out using tricycles, motorcycles and bicycles (Figure 4).

Transport costs range from 250 to 1000 FCFA depending on the distance. Forage plants are sold fresh in bales (Figure 5). The unit price of sale of forage species /boot varies from 100 to 150 FCFA depending on the season and the various holiday periods.

The average daily income is 750 FCFA and ranges from 18,750 to 37,500 FCFA per month or an average of 26,125 FCFA and ranges from 225,000 to 450,000 FCFA per year, or an average of 313,500 FCFA (Table 3)



**FIGURE 4: Collectors and transporters of forage plants using bicycles**



**FIGURE 5: Forage boots sold on livestock markets**

**TABLE 3**  
**ACTORS IN FORAGE MARKETING AND ECONOMIC LAND**

Title	Actors of the chain of sale of forage species									
	Single conveyors		Collectors / carriers		Single collectors		collectors / sellers		wholesalers	
	D	R	D	R	D	R	D	R	D	R
Average fodder transport cost / tricycle trip	250	1000	250	-	-	-	200	-	-	-
Average price for transporting 10 bunches of forage species	-	-	25	-	-	-	50	-	-	-
Average selling price of 10 fodder boots	-	-	-	1 300	-	-	250	1750	250	1150
Average daily income / actor of the sector	750		1275		400	1200	1500		900	
Average monthly income /sector actor (25 days)	18 750		31875		20 000		37500		22 500	
Average annual income /sector actor (300 days)	225 000		382 500		240 000		450 000		270 000	

*D - Expense; R - Income*

#### IV. DISCUSSION

The study of commercialized forage plants revealed nine (9) species belonging to five (5) genera and four (4) families. Studies have been conducted in some cities in Côte d'Ivoire and other cities in African countries on the sale of forage plants. In Mali, [8] and in Niger, [9] identified respectively 30 forage species belonging to 7 families in twenty-one markets in twenty-one markets and 34 species (14 families) in twenty-two points of sale in two cities. In Ivory Coast, [10] inventoried 37 forage species in 3 communes of the city of Abidjan. The number of species recorded is smaller and can be explained by the number of outlets and the cities where the surveys took place. Indeed, our investigations took place in the city of Daloa alone and only in three markets.

This study shows that the marketing of forage species in Daloa, which is not a new practice in Côte d'Ivoire, is practiced by men, either 100 percent of respondents. This result confirms those [11] realized on the marketing of spontaneous forage plants, especially trees and shrubs in the city of Bouaké, Côte d'Ivoire. But, it differs from those of [8] conducted in Burkina Faso, which revealed that 93 percent of the salespeople in the study sample were men and 7 percent women.

The results of the surveys show that the supply of the three livestock markets for forage crops is mainly provided by the periurban areas of the Gonaté-Daloa, Vavoua-Daloa and Issia-Daloa axes of the city of Daloa. Surveys indicate that the Gonaté-Daloa axis provides more than 55.89 percent of woody forage from fallows, old plantations and islands of natural formations.

Of the 45 stakeholders interviewed in the forage chain, most are nationals (82.22 percent) of neighboring countries (Mali and Burkina Faso). There is less Ivorian (17.18 percent) in this sector of activity. These results are consistent with the work of [12] on the Bouaké cattle market. This situation could be explained by the absence of pastoral tradition of Ivoirians populations, unlike nationals of neighboring countries, and also by the fact that Ivoirians are very little informed about the employment opportunities offered by the livestock sector.

The importance of commercial forage plants, as well as their economic benefits were recognized by the 45 actors interviewed in livestock markets in the city of Daloa. According to them, the average estimated financial income per day of an actor in the chain of sale of forage trees is 750 F CFA and varies between 18 750 to 37 500 F CFA per month, an average of 26 125 F CFA and oscillate between 225 000 to 450 000 F CFA, an average of 313 500 F CFA. As can be seen, the sale of forage plants is an essential source of income for many people who by this activity to meet their socio-economic needs as for any other job. These results are in agreement with the work of [3] which revealed a daily income of 875 F CFA, that is to say an average of 26 250 FCFA per month.

#### V. CONCLUSION

This work made it possible to know the forage sector, its actors involved in the marketing chain of ligneous fodder and the income derived from this sector. The forage species sold on the three (3) livestock markets in Daloa city are trees and shrubs

that belong mainly to the Moraceae families with five (5) species, either 55.56 percent. The supply of forage plants from the markets comes from the non-agglomerated areas of the city, fallows, islands of natural formations and old plantations especially on the Gonaté-Daloa axis. The commercialization of ligneous forage in the city is an economic activity which generates a significant profit for the actors of the sector and thus of the economic spin off per bundle of forage species sold varying on average 750 F CFA per day, either a monthly average of 26 125 F CFA. But, it is important to note that, although the harvesting and sale of woody forage plants generates substantial income, it is a source of degradation of natural formations already overexploited.

#### ACKNOWLEDGEMENTS

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