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Evaluation of biopolymeric particulate organic carbon of phytoplankton from El Rayah El Tawfiki and El Rayah El Menoufy of Nile Delta, Egypt

Howayda H. Abd El-Hady
National Institute of Oceanography and Fisheries,
101 Kasr Al Ainy, P.C. 11694, Cairo, Egypt
E-mail: Howayda999@hotmail.com

ABSTRACT

The concentration and composition of the biopolymeric particulate organic carbon (BPC) of the major phytoplankton components (proteins, carbohydrates and lipids) were measured in the two Nile Rayahs El Tawfiki and El Menoufy. BPC along El Rayah El Tawfiki (4.32-18.59 mgC/l) was associated to the high extent of the biopolymeric C of proteins (4.73-18.36 mgC/l). While the biopolymeric C of carbohydrates ranged between 0.08 to 0.27 mgC/l. The total concentration of biopolymeric C at El Rayah El Menoufy ranged from 6.19 to 15.42 mgC/l. The maximum concentrations of BPC detected at some sites of El Rayah El Menoufy were related to the higher biopolymeric carbon in both proteins and lipids. The present results showed a maximum positive correlation between both carbonate and bicarbonate of water body of El Rayah El Tawfiki and El Rayah El Menofy and BPC of phytoplankton (r= 0.9 and 1). BPC of phytoplankton was strongly related to the fraction of biopolymeric C associated with phytoproteins (r= 0.79). Variations in the estimated biochemical components of phytoplankton at the investigated Nile Rayahs followed the trend: Proteins> carbohydrates> lipids which indicated the highest protein contribution to C-BPC in both studied areas. Lipids/carbohydrates ratio in the two studied regions ranged between 0.55 and 0.57, which showed high nutritional value of organic matter in phytoplankton. Whereas, proteins/carbohydrates ratio ranged from 39.88 to 43.82, which indicated the aging of organic matter in phytoplankton. El Rayah El Tawfiki recorded higher energetic value (127.86 Kcal/l) than El Rayah El Menoufy (124.45 Kcal/l).This study demonstrated that fluctuation in phytoplankton composition due to the changes in carbonate and bicarbonate concentrations will have more impact on nutritional quality and biopolymeric carbon (BPC) of phytoplankton.

Key words: Phytoplankton, biopolymeric carbon, biochemical compounds, El Rayah El Tawfiki, El Rayah El Menoufy.

INTRODUCTION

El Rayahs El Tawfiki and El Menoufy are considered among the main irrigated canals of Nile Delta, Egypt (Talab *et al*., 2016). El Rayah El Tawfiki was constructed in 1889, it is regarded as one of the most ancient canals in Egypt, it's length is 192 km and irrigates 970,000 feddans. It crosses Qalyoubia, Dakahlia and Damietta Governorates. The average Water Quality Index (AWQI) at El Rayah El Tawfiki is about 70% which indicates the exposure to human activities (Shawky *et al*., 2013). El Rayah El Menoufy supplement from Rosetta Brach and crosses El Mansoura and Zifta cities, its length 170 Km (Ghannam *et al*., 2015).

Organic matter (OM) composition has been widely used to indicate the trophic state of the ecosystems (Cloern, 2001; Akhil *et al*., 2013; Renjith *et al*., 2013). Organic matter within water body is important in assessment of global biogeochemical cycles (Yamamuro, 2000; Goni

et al., 2003).The portion of OM and particulate biopolymeric organic carbon (BPC) has usually been assessed by the main biochemical contents (carbohydrates, proteins and lipids) of organic compounds (Fabiano *et al*., 1995; Dell'Anno *et al*., 2002; Pusceddu *et al*., 2009). Biochemical composition serves as a valid methodology for determination of the origin of organic matter (Colombo *et al*. 1996), also as an important tool to evaluate the nutritional quality of organic materials for consumer organisms (Rossi and Lardicci, 2002; Cividanes *et al*., 2002; Joseph *et al*., 2008). Ecological stoichiometry shows that the carbon content and the carbon-to-nutrient ratio of plant biomass are important biomarkers of ecological dynamics (Sudhakar and Premalatha, 2012). Carbon is the dominant element in organic matter, at moderate pH (>7) and temperatures > 30°C, the dominant form of CO_2 in water is bicarbonate (Sayre, 2010). CO_2, HCO_3^- and CO_3^{2-} can all serve as the source of carbon for algal growth and their production. Blue green algae growing autotrophically utilize the HCO_3^- and even CO_3^{2-} ion directly (Becker, 1994). The size and biochemical composition of phytoplankton communities can be controlled by carbon fixation through the environment (Armstrong *et al*., 2002).

This study aims to: (i) evaluate variability in biochemical components and nutritional value of phytoplankton at El Rayah El Tawfiki and El Rayah El Menoufy (ii) assess the quantity and quality of particulate biopolymeric organic carbon (BPC) of phytoplankton in the two Nile Rayahs.

MATERIALS AND METHODS

Water samples were collected from one site of River Nile (RN) and 7 selected sites from each El Rayah El Tawfiki and El Rayah El Menoufy from spring 2014 to winter 2015 (Fig.1).

Fig. (1). Map of the study area showing the sampling locations.

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1. Description of the study area

1. 1. El Rayah El Tawfiki:

El Rayah El Tawfiki extends for about 200 Km in length, with average width 40-50 m and average depth 2-3 m (Goher, 2015). It starts from Damietta Branch at El-Kanater El-Khayria city to the middle and the east of Delta heading north, parallel to the Damietta Branch until Mansoura city at site (T5). Then it extends to the east within Dakahlia Governorate to Manzalah city and branched at Mansoura city to the east direction. The maximum discharge of El Tawfiki is 18.56 M.m^3/day (Fathy *et al*., 2013**)**. This Rayah is characterized by existence of many drinking water stations and electric power plants on the two banks e.g. Banha drinking water station and other small water stations were used for villages, ezzabs and small towns which are heavily scattered on both sides of El Rayah. Water samples were collected from the beginning, middle and the end of El Rayah El Tawfiki at Mansoura, Dekerness and Manzalah cities, at sites (T1-T7) as shown in Table (1) and Figure (1).

1.2. El Rayah El Menoufy:

El-Rayah El Menoufy (about 170 Km in length with width ~50 m and depth ~3 m) starts from Rosetta Branch at El-Kanater El-khayria city and extends into the middle of Delta, breaking at Monifia, Dakahlia and El Gharbiah governorates, then heading north to Gamasa city and the south of Burullus Lake (Goher, 2015). El Rayah El Menoufy is characterized by the large number of small branches especially at El Monfia. At Zifta city (St. M5) a connected canal bearing large quantities of water from Damietta Branch discharge. The maximum discharge of El Rayah El Menoufy is 25 M.m^3/day (Fathy *et al*., 2013**)**. Water samples were taken from 7 sites (M1-M7) at El-Rayah El Menoufy (Table 1 and Fig. 1).

Table 1. Sampling sites at El Rayah El Tawfiki and El Rayah El Menoufy.

	Sites	Name	Latitude	Longitude	Distance from El-Kanater (Km)	Description
El Rayah El Tawfiki	T1	El-Kanater	30°11'46.58"	31° 7'55.98"	1-1.5	After line for railway line /very fast water /receives few wastewater.
	T2	Banha	30°28'24.0"	31°12'1.04"	40	In the front of Banha water plants /residential region in the right and left sides.
	T3	Met gamer	30°41'35.3"	31°16'50.2"	70	Beside agricultural way (Cairo-El-Mansorah)/between agricultural and residential regions.
	T4	Agga	30°54'23.12"	31°16'51.88"	100	Agricultural and residential regions in the right and left sides.
	T5	Mansoura	31°04'02.84"	31°25'02.13"	130	Before branching of El Rayah/ between agricultural and residential regions.
	T6	Dekerness	31° 5'38.65"	31°37'37.77"	155	Residential region in the west side and agricultural region in east side/low water level.
	T7	El-Manzalah	31°09'49.9"	31°56'09.5"	185	Static water/ receives the residues and wastes of El Rayah.
El Rayah El Menoufy	M1	El-Kanater	30°11'59.85"	31° 6'44.97"	1	Behind NIOF / receives few wastewater.
	M2	El-Khadra	30°20'15"	31° 02'55"	30	Middle of agricultural and residential regions.
	M3	Shebeen El-Koum	30°32'04.3"	31°00'48.3"	60	Receives the water from Damietta Branch.
	M4	El-Santa	30°43'44.50"	31° 7'28.88"	100	Under bridge/slaw water flow/ receives large amount of wastewater.
	M5	Zifta	30°47'26.15"	31° 9'16.12"	130	Receives the water from Damietta Branch
	M6	El-Mahalla	30°56'59.4"	31°09'21.4"	155	Middle of residential regions / receives few wastewater.
	M7	Belqas	31°07'59.9"	31°22'50.1"	185	Middle of agricultural regions.

2. Phytoplankton biochemical constituents and particulate biopolymeric organic carbon (BPC) analysis:

Water samples were collected and filtered through zooplankton net (100 μm mesh size) to separate macrozooplankton. The filtered water was refiltered on Whatman GF/F (0.7 μm pore diameter) fiber circles and the separated phytoplankton samples were transferred to the laboratory in ice tanks to determine their biopolymeric carbon (BPC). The total protein content was determined by Biuret method using bovine albumin as standard (David and Hazel, 1993). Carbohydrate contents were measured according to Phenol sulphuric acid hydrolysis method as described by Dubois *et al*. (1956), employing glucose as standard. Whereas the total lipid content was estimated by the Sulphophosphovanillin procedure utilizing cholesterol as calibration standard (Chabrol and Castellano, 1961). Phytoplankton caloric content was measured from the proximate biochemical composition, the relative caloric coefficients for proteins, carbohydrates and lipids were 5.56, 4.1 and 9.45 (Vollenweider, 2000). Concentrations of proteins, carbohydrates and lipids were converted to carbon equivalents using 0.40, 0.49 and 0.75 mgC/l conversion factors, respectively (Fabiano and Danovaro, 1994). The sum of protein carbon, carbohydrate C and lipid C is referred to as biopolymeric carbon (BPC) according to Fichez (1991) and Fabiano *et al*. (1995). The data of carbonate and bicarbonate were cited from Goher (2015) through the project of environmental status of the Nile Rayahs, Egypt.

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RESULTS AND DISCUSSION

Phytoplankton is responsible for about 50% of global primary production (Falkowski and Raven, 2007). The nutritional quality of phytoplankton available for aquatic herbivores depends mainly on their composition of lipid, protein and carbohydrate, which are affected by the changes in carbon as important environmental factors (Finkel *et al*., 2010). The present results illustrated that phytoplankton proteins exceeded in their values at most El Rayah El Tawfiki investigated sites (Av.= 22.22 mg/l) than River Nile (19.92 mg/l) except at stations (2 and 7) which represent the minimum contents of phytoplankton proteins (Table 2). Basiouny *et al*. (2008) illustrated that El-Rayah El Tawfiki and River Nile had almost the same pH, total dissolved solids and conductivity, which are in acceptable range, but more organic than that of River Nile, due to higher values of turbidity, total organic carbon (TOC) and humic acid. The values of phytocarbohydrates at El Rayah El Tawfiki ranged between 0.2 and 0.67 mg/l which is less than that obtained at River Nile (1.37 mg/l). The lipid peaked (0.41 mg/l) at st. (T5) as shown in Table (2). Particulate biopolymeric organic carbon (BPC) along El Rayah El Tawfiki (4.32-18.59 mgC/l) was associated to the high extent biopolymeric C of proteins (4.73-18.36 mgC/l). Synthesis of algal particulate organic carbon (POC) which is particulate protein amino acid (PPAA) carbon depends on carbon concentrations in the medium (Lohrenz *et al*., 1987).

The present results indicated that there was a significant relationship ($p \leq 0.05$) between *in situ* measurements of both CO_3 and HCO_3 and BPC of phytoplankton ($r^2 = 0.822$ and 0.842, respectively) in El Rayah El Tawfiki. While results of biopolymeric C of carbohydrates was ranged between 0.08-0.27 mgC/l. Carbohydrates are usually related to organic matter pre-eminently refractory in nature (Grémare *et al*., 2003; Pusceddu *et al*., 2009). The importance of the different biochemical contents of biopolymeric carbon might give information about the source and fate of sediment organic matter (Pusceddu *et al*., 2000; Fabiano *et al*. 2001).

Seasonal variations of phytoplankton biopolymeric C at El Tawfiki indicated that summer was the optimum for BPC of both proteins and carbohydrates (10.97 and 0.24 mgC/l, respectively), which may be related to higher CO_3 (Av.= 8.64 mg/l). The highest carbohydrate contents of phytoplankton at Ismailia Canal were recorded in summer (Abd El-Hady and Hussian, 2012).

Table 2. **Features of phytoplankton at El Rayah El Tawfiki based on carbon sequestration in proteins, carbohydrates, lipids and the particulate biopolymeric organic carbon (BPC).**

Sampling sites	Proteins (mg/l)	Biopolymeric C equivalent of proteins (mgC/l)	Carbohydrates (mg/l)	Biopolymeric C equivalent of carbohydrates (mgC/l)	Lipids (mg/l)	Biopolymeric C equivalent of lipids (mgC/l)	particulate biopolymeric organic carbon (BPC)
RN	19.92	9.76	1.37	0.55	0.37	0.28	10.59
T 1	26.91	13.19	0.40	0.16	0.24	0.18	13.53
T 2	9.66	4.73	0.60	0.24	0.32	0.24	5.21
T 3	25.13	12.31	0.43	0.17	0.35	0.26	12.74
T 4	25.44	12.47	0.67	0.27	0.30	0.22	4.32
T 5	19.16	9.39	0.42	0.17	0.41	0.31	9.87
T 6	37.47	18.36	0.30	0.12	0.15	0.11	18.59
T 7	11.76	5.76	0.20	0.08	0.23	0.17	6.01
Av.	21.93	10.89	0.55	0.17	0.30	0.21	11.27

There were a fluctuation in the results of proteins and lipids along El Rayah El Menoufy, where st. (M6) represents the optimum yield of both proteins and lipids, 29.87 and 0.55 mg/l,

which may be due to the increase of nutrients as a result of few wastewater discharge, whereas there was an obvious decrease in carbohydrate contents at all sites of El Menoufy than River Nile (Table 3). On the other hand, winter was the optimum for the determined biopolymeric carbon at El Rayah El Menoufy (10.91, 0.31 and 0.39 mgC/l for proteins, carbohydrates and lipids, respectively). The elevation in BPC in winter may be attributed to the increase of HCO_3 (150.16 mg/l). Algae can grow autotrophically utilizing the HCO_3^- and even CO_3^{2-} ion directly (Becker, 1994). Lipids responsible for oxidation processes of providing energy, through –C=O- bounds, than other biochemical compounds (Boëchat and Giani, 2000). Lipids constitute an important fraction of particulate OM in aquatic environments (Borsheim *et al*., 1999; Burdige *et al*., 2000).The results of the present study of total concentration of BPC along El Rayah El Menoufy ranged from 6.19 to 15.42 (mgC/l). The higher concentration of BPC at M6 related to increase biopolymeric carbon in both proteins and lipids (14.64 and 0.41 mgC/l) at this site. Proteins represent a significant portion of organic matter (Salas *et al*., 2015). Triacylglycerols (TAGs) or neutral lipids represent ~60% of the total dry weight of algae, more than 75% of the mass of TAGs is carbon, and thus TAGs represent rich sources of captured carbon in cells.

Data analyses illustrated that CO_3 is related significantly to lipids and BPb of phytoplankton at El Rayah El Menoufy (r^2= 0.633 and 1.00, respectively), while HCO_3 related to proteins, lipids and PBb (r^2= 0.999, 0.652 and 1.00, respectively). High incorporation of carbon into algal lipids occurred under nitrogen-deficient environment or during stationary growth phases of phytoplankton, whereas in nitrogen sufficient water carbon was highly incorporated into proteins (Lee *et al*., 2009).

Table (3). Features of phytoplankton at El Rayah El Menoufy based on carbon sequestration in proteins, carbohydrates, lipids and the particulate biopolymeric organic carbon (BPC).

Sampling sites	Proteins (mg/l)	Biopolymeric C equivalent of proteins (mgC/l)	Carbohydrates (mg/l)	Biopolymeric C equivalent of carbohydrates (mgC/l)	Lipids (mg/l)	Biopolymeric C equivalent of lipids (mgC/l)	particulate biopolymeric organic carbon (BPC)
RN	19.92	9.76	1.37	0.55	0.37	0.28	10.59
M 1	12.12	5.93	0.33	0.13	0.18	0.13	6.19
M 2	16.13	7.90	0.33	0.13	0.34	0.26	8.29
M 3	21.26	10.42	0.30	0.12	0.33	0.25	10.79
M 4	21.65	10.60	0.70	0.28	0.34	0.26	11.14
M 5	26.57	13.02	1.00	0.40	0.47	0.35	13.77
M 6	29.87	14.64	0.48	0.19	0.55	0.41	15.24
M 7	22.42	10.99	0.40	0.16	0.20	0.15	11.30
Av.	21.24	10.50	0.61	0.20	0.35	0.26	10.96

Variations in the estimated biochemical components of phytoplankton in the two Nile Rayahs followed the trend: Proteins> carbohydrates> lipids which indicated the highest protein contribution to C-BPC in the studied regions. Lipid/carbohydrates ratio in the two studied Rayahs ranged between 0.55 and 0.57, which showed high nutritional value of OM in phytoplankton. Lipids/carbohydrates ratio was used as a food quality index of OM (Gremare *et al*., 2002). Proteins/carbohydrates ratio in phytoplankton of the two Rayahs was 43.82-39.88, which indicated the aging of organic matter in phytoplankton (Salas *et al*., 2015). In phytoplankton amino acids occupied about 67% of organic carbon, while carbohydrates and lipids contain about 4% and 15%, respectively (Lee *et al*., 2000; Wakeham *et al*., 2000).

The caloric values of phytoplankton at the two studied regions have convergent values, where El Rayah El Tawfiki recorded the highest energetic value (127.86 Kcal/l) than that at El Menoufy (124.45 Kcal/l) as shown in Figure (2). The environmental variables interacted with the observed variations in energy content of the phytoplankton in fresh water lake, the quality of phytoplankton varied with energy content in the engulfing phytoplankton (Chattopadhyay, 2014).

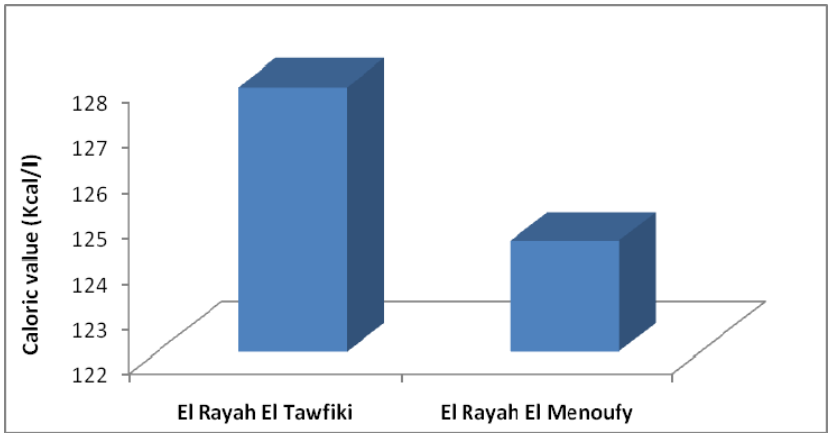

Fig. (2). Energetic value of phytoplankton (Kcal/l) at the two Nile Rayahs.

Canonical correspondence analysis (CCA) biplot diagram at the studied Nile Rayahs indicated that CO_3^{2-} and HCO_3^- have high effect on carbon captured by biochemical variables, where they recorded a high positive correlation with phytoplankton BPC, where r= 0.9 and 1 of El Rayahs El Tawfiki and El Menofy, respectively. A negative correlation with carbon equivalent of protein and carbohydrate were detected (Fig. 3). The analyses data suggested that increased carbon availability changes the biochemistry composition and thus nutritional quality and BPC of individual phytoplankton.

Microalgae is efficient to capture carbonate or bicarbonate as high as 90% in open ponds to fertilize algal growth (Sayre, 2010). One gram of algal biomass is produced by captured of 1.6-2.0g of carbon (Herzog and Golomb, 2004). Cyanobacteria and eukaryotic algae transport and use bicarbonate as a source of carbon dioxide (Spalding 2008: Jansson and Northen, 2010). The concentrations and biochemical composition of phytoplankton were pools of suspended organic particles in both studied El Rayahs. A positive relationship has been observed between BPC of phytoplankton and the fraction of biopolymeric C associated with phytoproteins (r= 0.79). The study of Pusceddu *et al*. (2010) confirmed that the primary productivity was a major factor controlling both quantity and nutritional quality of phytoplankton organic matter.

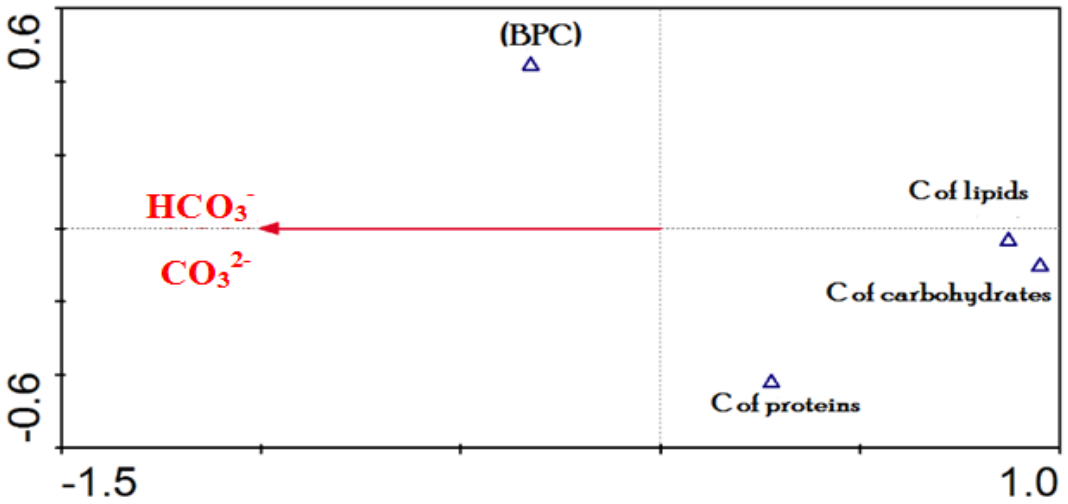

Fig. (3). Canonical Correspondence Analysis (CCA) for analyzed carbonate and bicarbonate with biopolymeric carbon in protein, carbohydrate, lipids and particulate biopolymeric organic carbon (BPC) of phytoplankton at the studied Nile Rayahs.

CONCLUSION AND RECOMMENDATION

The study indicated that the particulate biopolymeric organic carbon (BPC) of microalgae provides additional information on the origin, quality and characteristics of the organic matter available for food chain in El-Rayahs El Tawfiki and El Menofy of the River Nile. The results demonstrated that fluctuation in phytoplankton composition due to the changes of carbonate and bicarbonate concentrations will have more impact on nutritional quality and BPC of phytoplankton. More research in the field of molecular biology is needed to provide more information about the microalgae involved in production and recycling of carbon in fresh water environment.

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Howayda H. Abd El-Hady

تقدير عنصر الكربون العضوي البوليمر داخل الهائمات النباتيه فى كلاً من الرياح التوفيقى والرياح المنوفى لدلتا النيل، مصر

هويدا حسان عبد الهادى
المعهد القومى لعلوم البحار والمصايد، 101 القصر العينى، القاهره، مصر

المستخلص

تناولت الدراسه قياس الكربون العضوى البوليمر (BPC) من حيث التركيز والتكوين داخل الهائمات النباتيه لرياحيين أساسيين بنهر النيل وهم الرياح التوفيقى والرياح المنوفى حيث أنه يعد من المكونات الأساسيه والمقدره من محتوى الهائمات النباتيه من البروتينات والكربوهيدرات والدهون.

وقد أرتبطت قيم الكربون العضوى البوليمر (BPC) داخل هائمات الرياح التوفيقى (4.32 - 18.59 مجم كربون/لتر) بدرجه كبيره بقيم الكربون البوليمر المكون لبروتينات الهائمات النباتيه حيث تراوحت من 4.73 إلى 18.36 مجم كربون/لتر. بينما تراوحت قيم الكربون البوليمر للمواد الكربوهيدراتيه من 0.27 إلى 0.08 مجم كربون/لتر. وقدر التركيز الكلى للكربون العضوى البوليمر بالرياح المنوفى ب6.19 - 15.42 مجم كربون/لتر. وتعزى الزياده فى تركيز BPC الكربون العضوى البوليمر فى بعض محطات الرياح المنوفى إلى زيادة الكربون الحيوى البوليمر المكون للبروتينات والدهون داخل الهائمات النباتيه بهذه المحطات.

أوضحت النتائج الإحصائيه الترابط الإيجابى ما بين قيم الكربونات والبيكربونات لمياه الرياحين التوفيقى والمنوفى وبين قيم BPC الكربون العضوى البوليمر حيث بلغ معامل الترابط r = 0.9- 1 لكلاً من الرياحين، وأظهرت النتائج أيضا الترابط الشديد بين قيم BPC وبين قيم الكربون المكون للبروتينات حيث بلغ معامل الترابط r = 0.79.

قدرت قيم المحتوى الكيميائى الحيوى للهائمات النباتيه برياحين النيل على النحو التالى: المواد البروتينيه > المواد الكربوهيدراتيه > المواد الدهنيه مما يدل على مساهمة البروتين بدرجه كبيره فى التكوين الكربونى داخل الكربون العضوى البوليمر C-BPC بكلاً من الرياحين محل الدراسه. تراوحت نسبة الدهون/الكربوهيدرات من 0.55 - 0.57 بالرياحين، مما يشير إلى القيمه الغذائيه العاليه للمواد العضويه داخل الهائمات النباتيه. بينما بلغت نسبة البروتينات/الكربوهيدرات 39.88 - 43.82 مما يدل على قدم المواد العضويه داخل الهائمات النباتيه. وقد سجلت الهائمات النباتيه بالرياح التوفيقى قيم للسعرات الحراريه (127.86 كيلو سعر حرارى/لتر) أعلى من قيمها بالرياح المنوفى (124.45 كيلو سعر حرارى/لتر). أيدت الدراسه أن التغيرات فى تكوين الهائمات النباتيه نتيجة التغير فى تركيزات الكربونات والبيكربونات لها تأثير على الجوده الغذائيه والكربون العضوى البوليمر (BPC) للهائمات.