HUMIC-TYPE FLUORESCENCE FROM CHROMOPHORIC DISSOLVED ORGANIC MATTER, HUMIC ACIDS, AND CARBON NANOPARTICLES IN WATER

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ABSTRACT

CDOM (coloured or chromophoric dissolved organic matter) is present in all types of natural water and plays a significant role in its optical properties. The humic-type fluorescence band (emission in the blue region with a maximum within 400 to 500 nm) essentially depends both on the CDOM source and on the wavelength of the exciting radiation. Despite the long-term study of the properties of CDOM and humic substances (HS), which make up most of the CDOM, their spectral properties have not yet been explained. Difficulties arise due to the fact that because of the wide variety of these substances and their polydispersity, the exact composition of fluorophores is not known. Currently, there is an active search for individual components in the fluorescence spectra of CDOM fractions, humic preparations of various origin, as well as similar in chemical structure nano-sized particles of graphene oxide or so called carbon dots (CD). Assuming that all these substances could have similar groups of fluorophores, we compared the spectral properties of CDOM, fulvic acid (FA), humic acids (HA) of different genesis, and carbon dots. It has been revealed that the fluorescence properties of all studied samples depend significantly on the excitation wavelength. The analysis of emission/excitation properties allowed us to distinguish the following classes of substances with fluorophores similar in spectral characteristics: (a) CDOM of Karelian freshwater lakes - fulvic acid samples (humic-type fluorescence with a maximum at 440-460 nm and significant blue shift); and (b) CD - HA of coal origin (wavelength of humic-like emission at 500 to 515 nm, no blue shift). We propose the following chain of organic material transformation according to changes in degree of humification and optical properties: biopolymers → aquatic HS (CDOM and FA) → terrestrial HS (geopolymers) → fractionated carbon nanoparticles.

KEYWORDS

Humic substances, chromophoric dissolved organic matter (CDOM), natural water, humic acids, carbon dots, fluorescence spectroscopy

INTRODUCTION

Natural organic matter is present in all types of natural waters and soils and plays a significant role in their biogeochemistry and optical properties (1). Humic substances (HS) constitute from 50 to 90% of the organic matter of peat, coals, bottom sediments and inanimate matter of soil and aquatic ecosystems; however, their properties and composition strongly depend on the source of the matter. Along with this, HS represents a polydisperse system of variety of organic molecules of high molecular weight and unique composition; as a result, the study of the properties of HS is a challenging problem. Fluorescence spectroscopy is an effective method, which provides important information about components and sources of HS (2,3,4,5,6,7,8). Spectral analysis of HS allows not only to use them in oceanology to trace and distinguish the origin of waters (9,10,11) and in

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studying of interaction of HS and microorganisms (12), but also to investigate properties of industrial humic products (13), and humic and fulvic acids isolated from various resources (14).

Typical fluorescence spectra of natural CDOM consist of two bands with overlapping emission maxima (15,16). The intense and wide emission band in the blue range of spectra is known as humic-like fluorescence (λ_{max} ~ 400-500 nm); it depends both on the origin of the sample and on the excitation wavelength. The less intense band with emission maximum in the range of 300-350 nm (with excitation at 230 and 270-280 nm) is called protein-like due to the fact that such parameters are characteristic of the fluorescence of proteins, peptides and individual aromatic amino acids of tryptophan, tyrosine and phenylalanine. With the filtration of natural water by filters with pore size of 0.45 μm, the UV band of the fluorescence typically disappears. For this reason, only the humic-like band of fluorescence characterizes the CDOM of natural water samples.

Despite the long-term study of the properties of HS of various genesis, their spectral properties have not yet been explained in detail. Therefore, there is an active search for individual components in fluorescence spectra of CDOM (coloured or chromophoric dissolved organic matter) fractions in natural water, humic preparations of various genesis, as well as similar in chemical structure carbon nanoparticles. At present, there is a great scientific interest in studies of carbon nanoparticles or so called carbon dots (CD) – nanosized particles of graphite/graphene oxides. In the chemical process of oxidization, which is concomitant to decomposition of organic precursor – bulk graphite, graphene sheets, carbon nanotubes or onions, activated carbons from coal, wood and coconut (17), lactose (18), candles soot (19) – sheets of sp^2-hybridized graphene acquire excellent luminescent properties: intensive luminescence with high photostability, long lifetime and wide spectral range (20,21). Due to their optical properties, small sizes (1-10 nm), nontoxicity and biocompatibility, CD became promising nanoparticles for the usage as new fluorescence biomarker (22,23,24). At the same time, as was demonstrated by the authors of (25,26,27), the properties of these nanoparticles, first of all intensity and wavelength of luminescence, are dependent on the method of their synthesis, namely on the composition of chemical mixture, temperature and duration of the reaction, and on the final size of nanoparticles. The true nature of the CD fluorescence is not fully understood, but scientists think that it has quasi-molecular nature and, for its formation, a vicinity of oxidized parts of CD (of groups C–O, C=O and O=C–OH) to non-oxidized graphene bonds C=C is required (28,29).

The chemical composition and some similarity in the properties and origin let us presume that humic substances and carbon dots can have the same fluorophores, which, if confirmed, will clarify much the properties of both substances. For this purpose we conducted a comparative analysis of the luminescence properties of humic substances and carbon dots suspended in water. The objective of this study was to carry out a comparative analysis of the luminescence properties of humic substances and carbon dots suspended in water, and to reveal the contribution of individual fluorophores to the optical properties of the examined organic materials.

MATERIALS AND METHODS

This paper presents the results of spectroscopic studies of natural and commercial HS of various origins and CD. Moreover, a comparative analysis of their spectral characteristics is carried out.

Spectral characteristics of industrial humic substances were studied using samples of: i) fulvic acid (FA) standards of the International Humic Substances Society (IHSS) and ii) solutions of humic acids (HA) extracted from commercially available humic products of different producers. Initial humic products were manufactured by industrial technologies from different types of organic raw materials: fossil (brown coal BC–), peat (Pe–), leonardite (Le–) and lake bottom sediments (sapropel Sa–). Initial HS are soluble sodium or potassium humates that were produced, in general, by alkaline treatment of raw materials. Chemical characteristics of the studied compounds are given in (14).

To study natural DOM, samples of the surface layer of water were collected in August 2013 from the Onego Lake near the Kondopozhskaya Bay and three small lakes of marsh origin at the Kare-

lian coast of the White Sea: Elovoye, Verkhneye and Vodoprovodnoe lakes. Water samples were stored in sealed plastic bottles in the dark at +4°C until further spectral investigations. Filtration was carried out using disposable cellulose filter (Millipore) with 0.45 μm pore size.

Carbon dots used in this work were synthesized at the International Technology Center, Raleigh, United States, according to the method described in (27). The synthesis method consisted in the processing of the graphite layers in a mixture of sulfuric and nitric acids 3:1 at 128°C for 2 hours. Then, the resulting nanoparticles were extracted by centrifuging (5000 rpm for 10 min) and washed with water until the pH of the supernatant reached a value of 5. After that, the precipitate was dried out at vacuum at a 50°C temperature during the night and suspended in water for the preparation of the studied samples.

In this work, water suspensions of three CD samples were studied. The first suspension of carbon dots was a mixture of nanoparticles of different sizes (from 3 to 8 nm) with concentration of 0.01 g/L, hereinafter referred to as CD-mix. Two other samples of CD were synthesized the same way as the first one, but - by the use of 10K and 1K molecular filters - they were fractionated on CD with sizes of 5 nm and 3-4 nm, respectively. Due to their visible fluorescence colour under excitation by UV light, these samples were named CD-yellow and CD-blue, respectively. Water suspensions of CD-yellow and CD-blue were studied at concentrations of 0.02 g/L.

Fluorescence spectra of all studied samples were detected with a Solar CM 2203 luminescence spectrometer in standard quartz cuvettes having 10 mm optical path length. The spectrometer has dual monochromators for excitation and registration with automatically tunable filters that cut off the second order of the spectrum. The light source is a high pressure xenon lamp (150 W), which provides an almost continuous spectrum of radiation in the region of 200-800 nm. Fluorescence emission spectra were recorded with 1-nm accuracy for the 230-500 nm range of excitation wavelength, in steps of 10-50 nm. In the device the emission spectra were automatically corrected for wavelength-dependent sensitivity.

RESULTS

All samples of natural CDOM showed similar dependencies of the emission maximum wavelength λ_{max} on the excitation wavelength (Figure 1). Changing the excitation wavelength from 270 to 310 nm, there is a shift of the fluorescence maximum to short wavelengths (the so called blue shift of fluorescence). Conversely, the emission maximum of the humic-like fluorescence band constantly shifts to long wavelengths with increasing excitation wavelength ($\lambda_{ex} \geq 330$ nm).

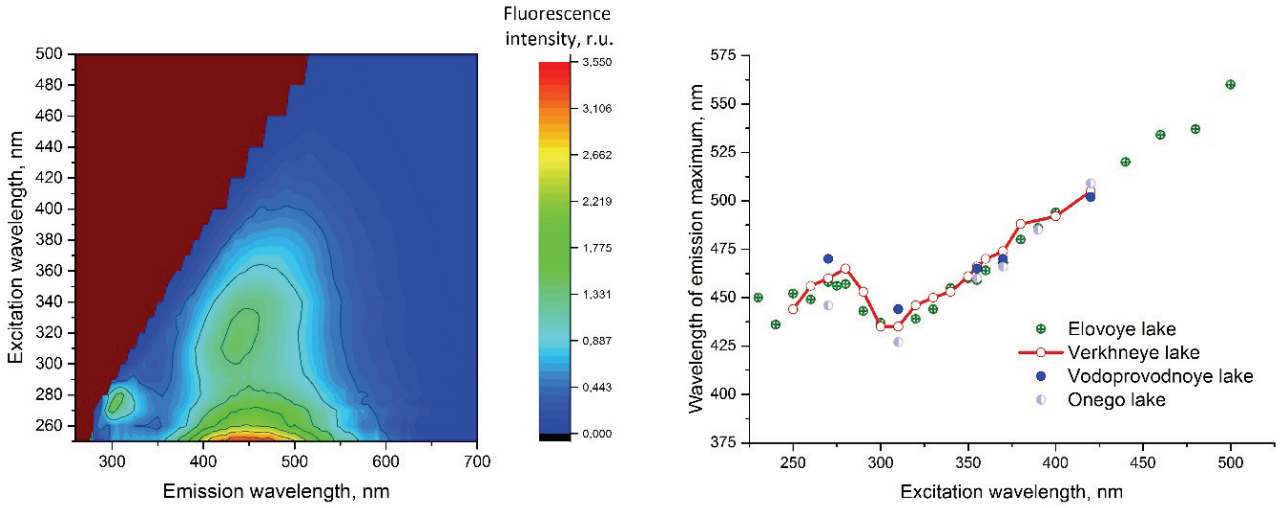

Figure 1: Excitation-emission matrix of CDOM from the Elovoye lake (left) and the dependencies of the emission maximum wavelength of CDOM of natural waters on the excitation wavelength (right).

Fluorescence spectra of HA samples in water under UV excitation have an emission with a maximum ranging within 490-520 nm and are excitation-dependent. They depend as well on the carbon

source of the used sample. The fluorescence maximum of HA extracted from the HS of peat origin is positioned at shorter wavelengths than that of HA extracted from the HS products from coal or sapropel (Figure 2, right). Moreover, when excited at 310 nm, peat-originating HA shows a small blue shift of the fluorescence emission, which is not typical for most industrial humic products. For example, HA samples extracted from sapropel (Sa–BigK) have no dependencies on the excitation wavelength varying within the UV range, while in case of coal originating HA samples, there is even a small shift of the maximum to the long-wavelength region (BC–EnNa, BC–EnK, BC–HumNa). The presence of a blue shift only in HA of peat origin, like in natural CDOM, can indicate a heterogeneity of fluorophores. In contrast, in HA samples of higher degree of humification the molecular composition is more homogeneous, which is reflected in their spectral characteristics.

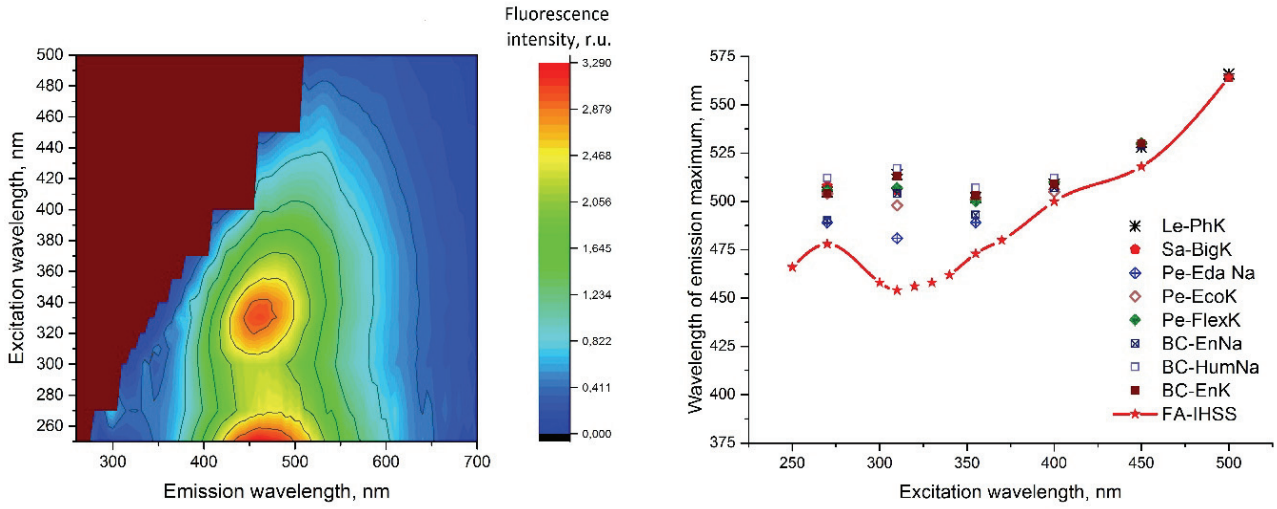

Figure 2: Excitation-emission matrix of FA IHSS (left) and the dependencies of the emission maximum wavelength of FA and HA of different origin on the excitation wavelength (right).

As can be seen from Figure 2, fluorescence spectra of FA sample (FA IHSS) are very different from all HA samples. Firstly, its fluorescence maximum, excited in the UV region, is positioned at shorter wavelengths than that of HA. Secondly, FA fluorescence exhibits a “blue shift” with excitation changing from 270 to 310 nm. The similar position of the fluorescence maximum and its dependence on the excitation wavelength leads us to conclude that fluorophore groups and fluorescence mechanisms are similar in the CDOM and FA samples.

The fluorescence of all carbon dots consists of several wide bands (Figure 3).

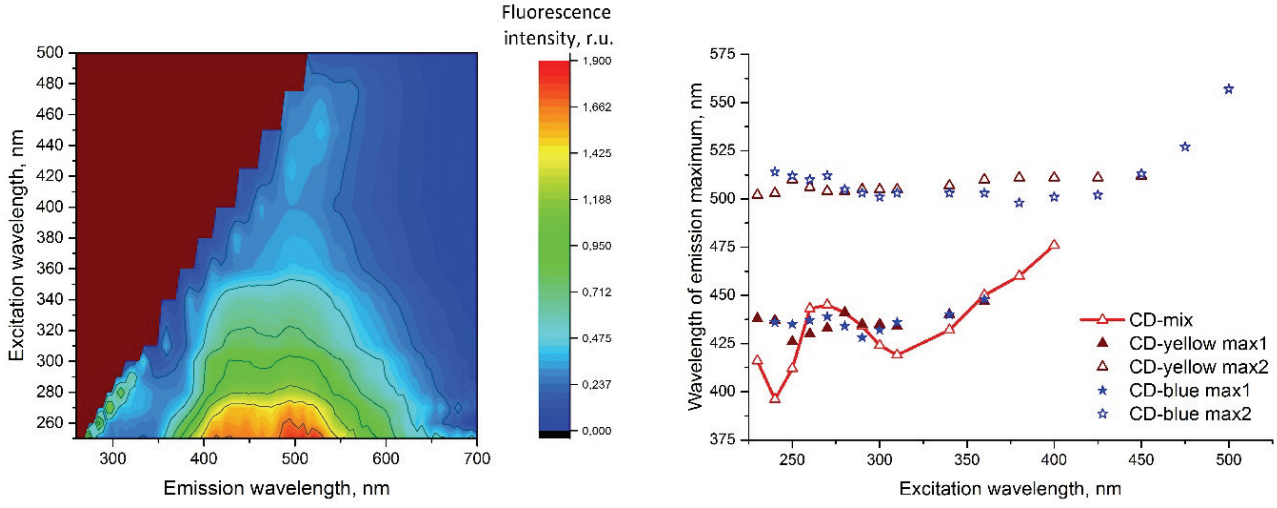

Figure 3: Excitation-emission matrix of CD-yellow (left) and the dependencies of the emission maximum wavelengths of CD on the excitation wavelength (right).

The CD-mix sample (mixture of nanoparticles of different sizes) manifests a first band in the UV range with maximum positioned at 310-330 nm with excitation at 230-290 nm. This UV emission of carbon dots resembles the protein-like fluorescence of CDOM. The second emission band of the CD-mix sample lies between 400-450 nm and has a pronounced dependence of the emission maximum on the excitation wavelength (large blue shift). This spectral behaviour of the visible emission band for the CD-mix sample and its "wavy" dependence of the emission maximum wavelength on the excitation wavelength remind the spectral properties of CDOM or FA samples (compare the red lines shown on Figures 1, 2 and 3).

For the two other samples, CD-yellow and CD-blue, the maxima of the fluorescence bands are positioned at 440-450 nm and 500-515 nm. However, no dependence of the fluorescence maximum on the excitation wavelength varying in the UV range can be found for both emission bands in the visible range. Similarities on the maximum wavelength of the second fluorescence band and its independence on the excitation wavelength, remind the characteristics of coal originating HA samples (BC–EnNa, BC–EnK, BC–HumNa). Therefore, we can assume the presence of similar fluorophore groups.

DISCUSSION

We describe above the similarities in dependence of emission maximum position on excitation wavelength in the UV range between CDOM, FA and CD-mix, as one group of samples, and coal-originating HA samples and size-fractionated CD, as the other group. However, for excitation above 350 nm (for humic-type emission around 420-450 nm) and 450 nm (emission band with a maximum at 505 nm) all studied samples showed the red-shifted fluorescence along with further increase of excitation wavelengths.

While features of the dependence of the fluorescence maximum position of CD-mix on excitation wavelength correspond to those of CDOM and FA, the position of fluorescence maxima of CD-yellow and CD-blue is nearly constant. Such a difference in the behaviour of studied dependencies approves the hypothesis that the fluorescence of CD does not originate from quantum effects but has a quasi-molecular origin (28,29) as discussed earlier. The red shift (30,31) and other features of the fluorescence of CD and, most likely, humic substances that accompany the change of excitation wavelength is explained by changes of prevailing fluorophores in samples. Suspensions of CD-yellow and CD-blue have the same position of the fluorescence maxima (Figure 3) but different intensity of the corresponding bands. With CD-yellow the band with the 515 nm maximum dominates over the band with the 435 nm maximum, which results in a yellow colour of the sample. Alternatively, with CD-blue the band with the 435 nm maximum dominates over the band with the 515 nm maximum, resulting in a blue colour. In that way, fluorophores of our samples of carbon dots turned out to be "tied" to the nanoparticles sizes. After the passage of CD-mix through molecular filters of different sizes: 1) for fractions CD-yellow and CD-blue of CD-mix the dependence of the position of fluorescence maximum on excitation wavelength disappeared, 2) a different ratio of fluorescent band intensities in CD-yellow and CD-blue occurs, indicating the different ratio of the corresponding fluorophores. The CD-mix sample, apparently, has additional fluorophores, which provide shifts of the maximum of fluorescence, that were "cut out" during filtering.

The similarities in properties of CDs and humic substances is reaffirmed by imnvestigations on four HS samples which demonstrate that all studied HS samples contained large quantities of carbon-based dots represented by small-sized graphene oxide nano-sheets with heights less than 1 nm and lateral sizes less than 100 nm (32). This finding of carbon dots in HS gave us new insight into HS optical properties.

Based on the spectral data obtained in this work and on the literature data discussed above, we propose the following formation chain of organic material according to their degree of humification and optical properties: biomolecules and biopolymers (aromatic amino acids, proteins and phenols) as HS precursors → aquatic HS with major part of FA (CDOM, FA) → HS of soil, peat, coal (as geopolymers) → carbon nanoparticles.

CONCLUSIONS

The fluorescence properties of the following substances were analysed: i) CDOM in natural water, ii) humic products of different origin and iii) CD (nanosized graphene oxide particles). A similarity in dependencies of the positions of emission maxima on the excitation wavelength was found between CDOM, FA and CD-mix (mixture of nanoparticles of different sizes), on one side, as well coal-originating HA samples and fractionated CD (according to their size), on the other side. These findings indicate similar fluorophores in those two groups of carbonized materials. As a consequence, the following changes of optical properties of the substances can be suggested: aromatic amino acids and proteins (as biopolymers) → aquatic HS (CDOM and FA) → terrestrial HS (geopolymers) → fractionated carbon nanoparticles.

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REFERENCES

1 Nebbioso A & A Piccolo, 2013. Molecular characterization of dissolved organic matter (DOM): a critical review. Analytical and Bioanalytical Chemistry, 405(1): 109-124 http://doi.org/10.1007/s00216-012-6363-2

2 Boyle E S, N Guerriero, A Thiallet, R Del Vecchio & N V Blough, 2009. Optical properties of humic substances and CDOM: relation to structure. Environmental Science & Technology, 43: 2262-2268. http://doi.org/10.1021/es803264g

3 Coble P G, 1996. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Marine Chemistry, 51(4): 325-346. http://doi.org/10.1016/0304-4203(95)00062-3

4 Coble P G, S A Green, N V Blough & R B Gagosian, 1990. Characterization of dissolved organic matter in the Black Sea by fluorescence spectroscopy. Nature, 348: 432-435. http://doi.org/10.1038/348432a0

5 Patsaeva S & R Reuter, 1995. Spectroscopic study of major components of dissolved organic matter naturally occurring in water. Global Process Monitoring and Remote Sensing of the Ocean and Sea Ice, 2586: 151-160. http://doi.org/10.1117/12.228618

6 Her N, G Amy, D McKnight, J Sohn & Y Yoon, 2003. Characterization of DOM as a function of MW by fluorescence EEM and HPLC-SEC using UVA, DOC, and fluorescence detection. Water Research, 37(17): 4295-4303. http://doi.org/10.1016/S0043-1354(03)00317-8

7 Trubetskoi O A & O E Trubetskaya, 2017. Three-dimensional fluorescence analysis of chernozem humic acids and their electrophoretic fractions. Eurasian Soil Science, 50(9): 1018-1024. http://doi.org/10.1134/S1064229317090083

8 Khundzhua D A, S V Patsaeva, O A Trubetskoj & O E Trubetskaya, 2017. An analysis of dissolved organic matter from freshwater Karelian lakes using reversed-phase high-performance liquid chromatography with online absorbance and fluorescence analysis. Moscow University Physics Bulletin, 72(1): 68-75. http://doi.org/10.3103/S002713491701009X

9 Gonçalves-Araujo R, M A Granskog, A Bracher, K Azetsu-Scott, P A Dodd & C A Stedmon, 2016. Using fluorescent dissolved organic matter to trace and distinguish the origin of Arctic surface waters. Scientific Reports, 6: 33978. http://doi.org/10.1038/srep33978

10 Yamashita Y, F Hashihama, H Saito, H Fukuda & H Ogawa, 2017. Factors controlling the geographical distribution of fluorescent dissolved organic matter in the surface waters of the Pacific Ocean. Limnology and Oceanography, 62(6): 2360-2374. http://doi.org/10.1002/lno.10570

11 Drozdova A N, S V Patsaeva & D A Khundzhua, 2017. Fluorescence of dissolved organic matter as a marker for distribution of desalinated waters in the Kara Sea and bays of Novaya Zemlya archipelago. Oceanology, 57(1): 41-47. http://doi.org/10.1134/S0001437017010039

12 Khundzhua D A, S V Patsaeva, V A Terekhova & V I Yuzhakov, 2013. Spectral characterization of fungal metabolites in aqueous medium with humus substances. Journal of Spectroscopy, 2013: 538608. http://doi.org/10.1155/2013/538608

13 Gosteva Yu O, A A Izosimov, S V Patsaeva, O S Yakimenko & V I Yuzhakov, 2012. Fluorescence of aqueous solutions of commercial humic products. Journal of Applied Spectroscopy, 78(6): 884-891. http://doi.org/10.1007/s10812-012-9548-8

14 Yakimenko O, D Khundzhua, A Izosimov, V Yuzhakov & S Patsaeva, 2016. Source indicator of commercial humic products: UV-vis and fluorescence proxies. Journal of Soils and Sediments, 16: 1-13. https://doi.org/10.1007/s11368-016-1528-9

15 Wang Z, J Cao & F Meng, 2015. Interactions between protein-like and humic-like components in dissolved organic matter revealed by fluorescence quenching. Water Research, 68: 404-413. http://doi.org/10.1016/j.watres.2014.10.024

16 Stolpe B, Z Zhou, L Guo & A M Shiller, 2014. Colloidal size distribution of humic- and protein-like fluorescent organic matter in the northern Gulf of Mexico. Marine Chemistry, 164: 25-37. http://doi.org/10.1016/j.marchem.2014.05.007

17 Qiao Z A, Y F Wang, Y Gao, H W Li, T Y Dai, Y L Liu & Q S Huo, 2010. Commercially activated carbon as the source for producing multicolor photoluminescent carbon dots by chemical oxidation. Chemical Communications, 46: 8812-8814. http://doi.org/ 10.1039/C0CC02724C

18 Chen Z B, J Wang, H Miao, L Wang, S Wu & X M Yang, 2016. Fluorescent carbon dots derived from lactose for assaying folic acid. Science China Chemistry, 59(4): 487-492. http://doi.org/10.1007/s11426-015-5536-1

19 Liu H, T Ye & C Mao, 2007. Fluorescent carbon nanoparticles derived from candle soot. Angewandte Chemie, 46(34): 6473-6475. http://doi.org/ 10.1002/anie.200701271

20 Baker S N & G A Baker, 2010. Luminescent Carbon Nanodots: Emergent Nanolights. Angewandte Chemie, 49(38): 6726-6744. http://doi.org/10.1002/anie.200906623

21 Dolenko T A, S A Burikov, K A Laptinskiy, J M Rosenholm, O A Shenderova & I I Vlasov, 2015. Evidence of carbon nanoparticle – solvent molecule interactions in Raman and fluorescence spectra. Physica Status Solidi A, 212(11): 2512-2518. http://doi.org/10.1002/10.1002/pssa.201532203

22 Yang S T, L Cao, P G J Luo, F S Lu, X Wang, H F Wang, M J Meziani, Y F Liu, G Qi & Y P Sun, 2009. Carbon dots for optical imaging in vivo. Journal of the American Chemical Society, 131(32): 11308-11309. http://doi.org/10.1021/ja904843x

23 Cao L, X Wang, M J Meziani, F S Lu, H F Wang, P J G Luo, Y Lin, B A Harruff, L M Veca, D Murray, S-Y Xie & Y-P Sun, 2007. Carbon dots for multiphoton bioimaging. Journal of the American Chemical Society, 129(37): 11318-11319. http://doi.org/10.1021/ja073527l

24 Prabhakar N, T Nareoja, E von Haartman, D Sen Karaman, S A Burikov, T A Dolenko, S Jaikishan, V Mamaeva, P E Hanninen, I I Vlasov, O A Shenderova & J M Rosenholm, 2015. Functionalization of graphene oxide nanostructures improves photoluminescence and facilitates their use as optical probes in preclinical imaging. Nanoscale, 7: 10410-10420. http://doi.org/10.1039/C5NR01403D

25 Lu J, J X Yang, J Z Wang, A L Lim, S Wang & K P Loh, 2009. One-pot synthesis of fluorescent carbon nanoribbons, nanoparticles, and graphene by the exfoliation of graphite in ionic liquids. ACS Nano, 3(8): 2367-2375. http://doi.org/10.1021/nn900546b

26 Peng J, W Gao, B K Gupta, Z Liu, R Romero-Aburto, L Ge, L Song, L B Alemany, X Zhan, G Gao, S A Vithayathil, B A Kaipparettu, A A Marti, T Hayashi, J J Zhu & P M Ajayan, 2012. Graphene Quantum Dots Derived from Carbon Fibers. Nano Letters, 12(2): 844-849. http://doi.org/10.1021/nl2038979

27 Hens S C, W G Lawrence, A S Kumbhar & O Shenderova, 2012. Photoluminescent nanostructures from graphite oxidation. Journal of Physical Chemistry C, 116(37): 20015-20022. http://doi.org/10.1021/jp303061e

28 Shang J, L Ma, J Li, W Ai, T Yu & G G Gurzadyan, 2012. The origin of fluorescence from graphene oxide. Scientific Reports, 2, 792. http://doi.org/10.1038/srep00792

29 Galande C, A D Mohite, A V Naumov, W Gao, L Ci, A Ajayan, H Gao, A Srivastava, R B Weisman & P M Ajayan, 2011. Quasi-molecular fluorescence from graphene oxide. Scientific Reports, 1: 85. http://doi.org/10.1038/srep00085

30 Cushing S K, M Li, F Huang & N Wu, 2014. Origin of strong excitation wavelength dependent fluorescence of graphene oxide. ACS Nano, 8(1): 1002-1013. http://doi.org/10.1021/nn405843d

31 Dolenko T A, S A Burikov, A M Vervald, A A Khomich, O S Kudryavtsev, I I Vlasov & O A Shenderova, 2016. Observation of the “red edge” effect in the luminescence of water suspensions of detonation nanodiamonds. Journal of Applied Spectroscopy, 83(2): 294-297. http://doi.org/10.1007/s10812-016-0284-3

32 Dong Y, L Wan, J Cai & G Chen, 2015. Natural carbon-based dots from humic substances. Scientific Reports, 5: 10037. http://doi.org/10.1038/srep10037