Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 August 2019 doi:10.20944/preprints201908.0221.v1

Review

Low-intensity Exposures via Luminescent Bioassays of Different Complexity: Cells, Enzyme Reactions and Fluorescent Proteins

Nadezhda S. Kudryasheva [1,2]* and Ekaterina S. Kovel [1,3]

[1] Institute of Biophysics SB RAS, FRC KSC SB RAS, Krasnoyarsk, 660036, Russia; n-qdr@yandex.ru (N.S.K.); kkovel@yandex.ru (E.S.K.);
[2] Siberian Federal University, Krasnoyarsk, 660041, Russia;
[3] Institute of Physics SB RAS, FRC KSC SB RAS, Krasnoyarsk, 660036, Russia
* Correspondence: n-qdr@yandex.ru; Tel.: +7-3912-494-242

Abstract: Current paper reviews applications of luminescence bioassays for monitoring low-intensity factors, namely, radioactivity of different types (alpha, beta and gamma), and bioactive compounds (humic substances and fullerenols). Luminescence intensity is taken as a physiological parameter of luminous organisms. High rates of luminescence response can provide (1) a proper number of experiments under comparable conditions and, therefore, proper statistical processing, with this being highly important for 'noisy' low-dose exposures; (2) non-genetic, i.e. biochemical and physicochemical mechanisms of cellular response, in accordance to "exposome" concept. Bioassays based on luminous marine bacteria, their enzymes, and fluorescence coelenteramide-containing proteins were used to compare results of low-intensity exposures at cellular, biochemical and physicochemical levels, respectively. Results of the cellular exposures were discussed in terms of hormesis concept. Bioluminescence time dependence under low-dose radiation exposures corresponded to hormesis or threshold models; no bioluminescence monotonic dependency on intensity of exposure (dose rate, radioactivity, concentration) was found. Bioluminescence activation and absence of its dependency on intensity of exposure can be accepted as features of cellular adaptive response. Changes of biological luminescence were analyzed and discussed for bioassays of lower organization level – enzymes and florescent protein.

Keywords: luminescence bioassays; bacterial cells, enzymes; fluorescent protein; low-intensity factors; hormesis; radiation; bioactive compounds; antioxidant activity

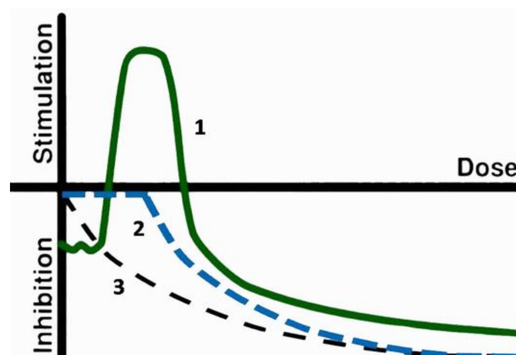
---

1. Introduction

All biological objects on the Earth are exposed to low-intensity factors – radioactive, chemical, and electromagnetic. Currently, accumulation of evidence of the inhibitory and activating effects of these factors is ongoing. Activation of physiological functions of organisms is associated with term "hormesis", which implies a favorable biological response to the low impact of toxins and other stressors. This term was introduced by Southam and Ehrlich in 1943 [1-2] based on observations that "extracts from the Red Cedar tree enhanced the metabolism of fungal species". Over the past decades, an exponential increase in citation for hormesis in the biomedical community has been observed [2-5]. Hormesis model is based on non-linear dependence of effect on dose of toxic compounds, as shown schematically in Figure 1. Hormesis is considered as the basic model; two other models (linear and threshold) can be considered as particular cases of hormesis [2,5].

© 2019 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 August 2019 doi:10.20944/preprints201908.0221.v1



41 **Figure 1.** Scheme of dose-effect models: 1 – hormesis, 2- threshold, 3- linear.

42 There exist evidences that hormesis is highly generalized phenomenon; it does not depend on
43 the level of biological organization (cells, organs, or organisms). There exists an urgent need to
44 understand and predict responses of organisms to low-intensity exposures. This understanding
45 should be based on molecular mechanisms of these effects, which are not clear yet. We suppose that
46 simple bioassay systems, such as cells and enzymes, will allow understanding the low-intensity
47 effects at cellular and biochemical levels, respectively. The next stage of investigation should be
48 concerned with the level of physicochemical processes (energy, electron or hydrogen transfer) and
49 application of the simplest bioassay systems.
50 Current review considers application of luminescence bioassay systems of different level
51 organization (cells, enzyme reactions, and fluorescent proteins) for study mechanisms (cellular,
52 biochemical, and physicochemical, respectively) of low-intensity exposures.
53 Luminescence feature of bioassay systems provide a proper registration of biological responses.
54 Luminescence intensity is a testing physiological parameter of these bioassays. The advantages of the
55 luminescence are high rates of analysis (down to 1-3 min), ease of use, high sensitivity, and
56 availability of instruments and reagents. Since the luminescent type of registration is not time
57 consuming, it provides a lot of experimental results under comparable conditions, with this being
58 essential for their statistical processing. This advantage is very important for biological analyses,
59 which are usually characterized by lower reproducibility than chemical or radiometric assays. This
60 advantage is of particular importance in study of the low-intensity exposures that are usually can be
61 described in terms of "stochastic effects" [6]. Additionally, quick response can contribute to
62 investigation of non-genetic mechanisms of low-intensity exposures.
63 The most known luminescent cellular bioassay is based on luminous marine bacteria. Bacterial
64 luminescence is sensitive to toxic compounds; this is a reason why the marine bacterium has been
65 used for several decades to assess environmental toxicity [7-13].
66 Current tendency to simplification of bioassay systems resulted in development of enzymatic
67 assays. As opposite to the cell-based assay, enzymatic assays estimate rates of biochemical reactions
68 under toxicant influence. Enzymatic bioluminescence assay based on the bacterial enzymes,
69 progresses from early 90-s [14-17]. Solid immobilized enzymatic and bacterial preparations develop
70 now as a basis for bioluminescent biosensors [14,17-20]. Classification of toxic effects was suggested
71 first in [21] and developed later in [22-25]. This classification describes (1) physicochemical, (2)
72 chemical, and (3) biochemical processes in the bioluminescence assay systems at exposure to toxic
73 compounds.
74 Advanced experience in study the toxic effects on bioluminescence of bacteria and their enzymes
75 is one more advantage of their application for evaluation the effects of low-intensity exposures.
76 Biochemical and physicochemical approaches contribute to non-genetic aspect of toxic and
77 adaptive effects. These approaches are applicable in frames of novel "exposome" concept, where
78 "exposome complements the genome and encompasses the totality of environmental non-genetic
79 exposures" [26-28]. Exposome concept was originated as a challenge in molecular epidemiology [29]
80 and concerned with human exposures. Application of simple model organisms and biochemical

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 August 2019 doi:10.20944/preprints201908.0221.v1

systems might provide human exposure sciences with fundamental support basing on molecular, physicochemical, biochemical, and cellular investigations.

This review analyzes results of application the simplest luminescence bioassays (luminous marine bacteria, their enzymes, and fluorescent coelenetamide-containing proteins) to study (i) the effects low-dose radiation of alpha, beta and gamma type, and (ii) antioxidant effects of bioactive compounds of natural and artificial origination – humic substances and fullerenols, respectively.

Next section of the review, Section 2, justifies an application of the coelenteramide-containing fluorescent protein as a simplest multicolor bioassay based on physicochemical process in the protein complex. Section 3 discusses application of luminescence bioassays in study the low-dose radiation effects. Section 4 presents low-concentration antioxidant effects of bioactive compounds.

2. Coelenteramide-Containing Fluorescent Protein as a Simplest Multicolor Fluorescent Bioassay for low-intensity exposures

Main structural components of fluorescent proteins are polypeptide and aromatic fluorophore; the latter is responsible for light emitting. The most important representative of fluorescent proteins is Green Fluorescent Protein (GFP). It was isolated in 1962 from jellyfish *Aequorea victoria* by American scientist O. Shimomura. In 2008 Simimura has got a Nobel Prize for the discovery and development of GFP. A series of fluorescent proteins of different color, homologues to GFP, are known now [30-31]. They are widely used in medical and biological research for labeling individual molecules, intracellular structures, living cells, organs and whole organisms in order to visualize intracellular processes [32-33].

Coelenteramide-containing proteins are the other group of fluorescent proteins. "Discharged aequorin", a representative of this group, was isolated and studied by Prof. Shimomura too, simultaneously with GFP. He called it Blue Fluorescent Protein (BFP). This group differs from the GFPs with fluorophore formation: the fluorophore of GFPs is formed by amino acid residues, while the fluorophore of BFP is a coelenteramide molecule (Figure 2). As opposed to GFPs, the coelenteramide-containing proteins are not widely used, and their potential as color fluorescent biomarkers is not evaluated yet.

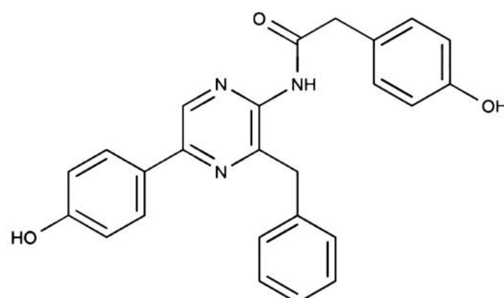
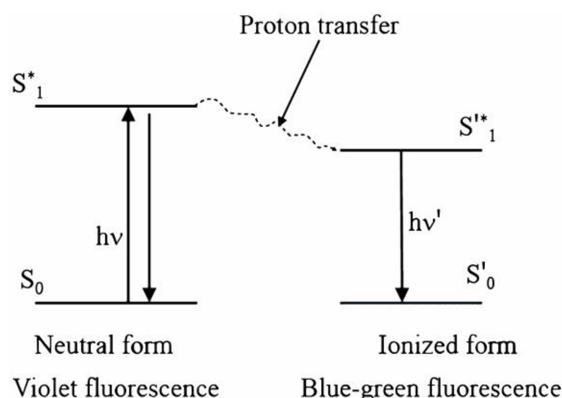


Figure 2. Chemical structure of coelenteramide molecule.

Coelenteramide-containing proteins are known to be products of bioluminescent reactions of marine coelenterates. The reactions are Ca-dependent, and this is a basis for their biomedical application [34-35]. Biochemical and photophysical mechanisms of the bioluminescence reactions [36-41] and spectral characteristics of their products - coelenteramide-containing proteins [42-49] are under intensive investigations now.

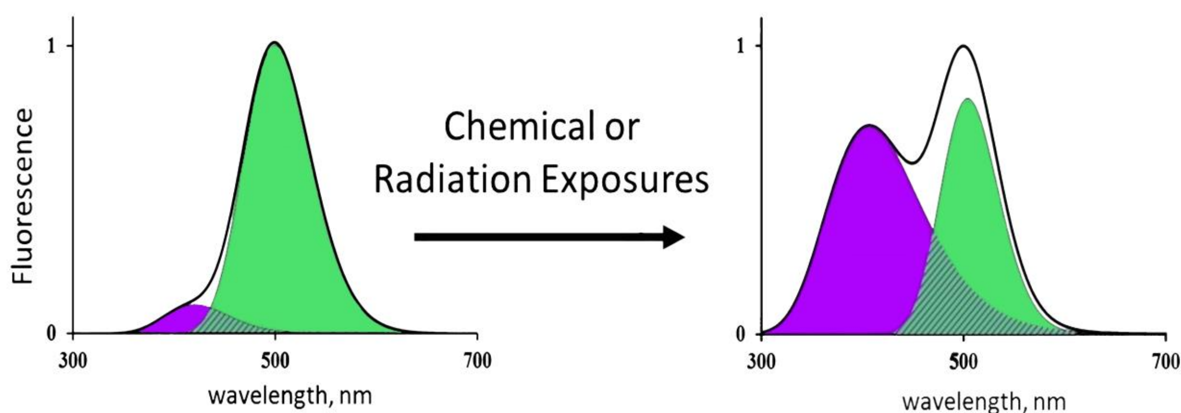
We suggested application of coelenteramide-containing proteins as simplest bioassay system basing on their ability to change fluorescence color under exposure to chemical toxicants, radiation, and other destructive factors [50]. Peculiarities of this bioassay are concerned with photobiophysical properties of the protein complex. Coelenteramide (CLM) molecule is a photochemically active compound; its photoexcitation initiates a proton transfer out (Figure 3). Fluorescence color of CLM and deprotonated CLM differ (violet and green spectral regions, respectively) (Figures 3,4).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 August 2019 doi:10.20944/preprints201908.0221.v1



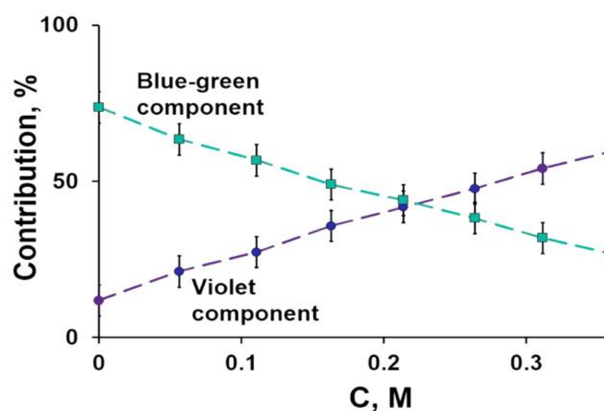
120 **Figure 3.** Chemistry in electron-excited states in coelenteramide molecule. Jablonslki diagrams of
121 two forms of colenteramide.

122 Any destructive exposures can decrease the efficiency of the photochemical reaction (proton
123 transfer out) in the protein complex, change contributions of fluorescence components, and hence,
124 fluorescence color. Therefore, CLM-containing protein based bioassay provides relations of a toxic
125 effect with a primary physicochemical process – proton transfer. Figure 4 shows schematically a
126 result of the destructive exposures: chemical or radioactive exposures can change contributions of
127 colored components to the fluorescence spectra of CLM-containing protein.



128 **Figure 4.** Change of fluorescence spectra of CLM-containing fluorescence proteins exposed to
129 chemical agents or radiation.

130 Figure 5 presents fluorescent component contributions to CLM-containing protein spectra with
131 glycerol taken as an example of chemical agent.



132 **Figure 5.** Contributions of spectral components to fluorescence spectra of CLM-containing protein at
133 different concentrations of glycerol, C [51].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 August 2019 doi:10.20944/preprints201908.0221.v1

Therefore, variations of fluorescence color of CLM-containing proteins are the result of destructive protein exposures; toxicity monitoring, in this case, is concerned with the changes in protein structure; the toxicity evaluation takes place via efficiency of primary photochemical processes of proton transfer. Low-intensity exposures of CLM-containing proteins to radiation or chemical agents are of fundamental interest.

3. Luminescence bioassays as tools for study low-dose radiation effects

The intensity of studying radiobiological low-dose effects has been growing since the 70s [52-56], including effects on microorganisms [57-59]. Radiation hormesis is intensively discussed. The first radiation hormesis tutorial was written by Luckey in 1980 [60].

Luminous marine bacteria have been applied to monitor low-dose radiation effects for about one decade [13,15]. For this period, effects of alpha- and beta- emitting radionuclides americium-241, uranium-235+338, and tritium, as well as gamma radiation were investigated [13,15-16,61-63]. It was shown that the bacterial bioluminescence response to radionuclides americium-241 and tritium includes three stages: (1) threshold, (2) activation, and (3) inhibition. We have chosen these two radionuclides for presentation here due to their radioecological significance: both radionuclides are accumulated in environment now. Tritium is a by-product of a lot of radiochemical reactions in nuclear industry; americium-241 is a by-product of a plutonium decay with high radiation lifetime (432,6 years).

Bacterial bioluminescence kinetics in solution of americium-241, alpha-emitting radionuclide of high specific radioactivity, is presented in Figure 6.

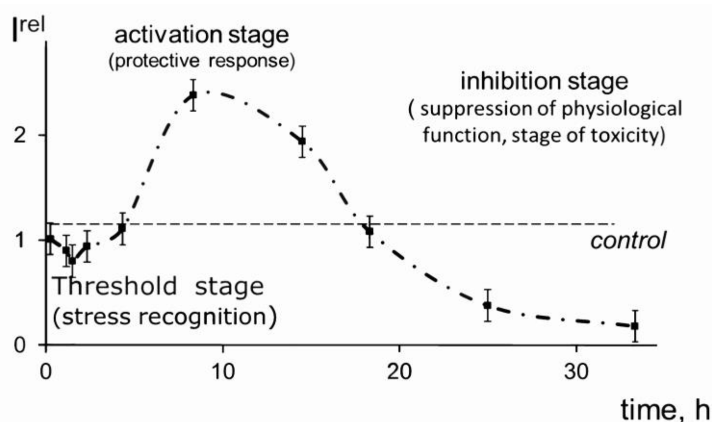
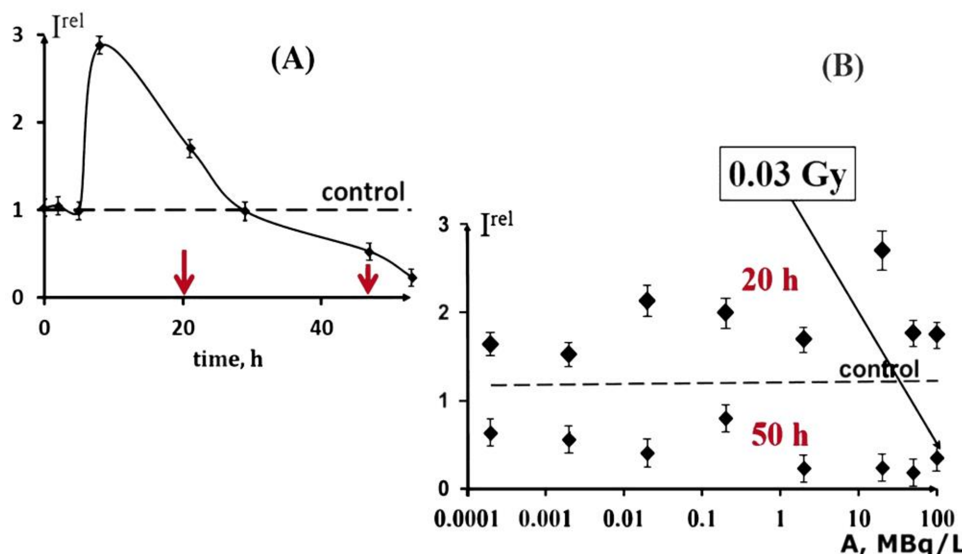


Figure 6. Bioluminescence kinetics of bacteria in solution of americium-241, 3 kBq/L.

Similar kinetic changes were obtained at exposure to beta-emitting radionuclide, tritium [16,63]. Figure 7A reveals the same three stages in bacterial luminescence response, with activation included. Hence, activation is a main peculiarity of the response of bacteria to low-dose radiation of alpha and beta emitting radionuclides - americium-241 and tritium, respectively. The responses can be discussed in terms of “radiation hormesis”, as well as “protective response of organisms”.

Additionally, independence of bioluminescence bacterial response on tritium activity concentration was found for low-dose exposures. To demonstrate this peculiarity, the time of exposure to tritium was fixed 20 and 50 hours, corresponding to activation and inhibition stages of bioluminescence (Figure 7A). The bioluminescence intensity at different concentrations of tritium is presented in Figure 7B for 20 and 50 h exposure times. It is seen here that the monotonic dependence is absent in a wide interval of activity concentrations of tritium – five orders. The result can be explained in terms of adaptation ability of the bacterial cells. It should be paid attention that the conventional limit of low-dose interval (0.1 Gy) was not exceeded in this experiment.



168 **Figure 7.** Effect of tritiated water on bioluminescence of bacteria. (A) Bioluminescence kinetics of
169 bacteria in tritiated water, 2 MBq/L; (B) bioluminescence intensity vs. activity concentration of
170 tritiated water, A, at 20 and 50 h exposures.

171 Activation of the bacterial bioluminescence by tritium was demonstrated in a series of
172 experiments. Bi-phasic dependence (activation+ inhibition) was found in [16,63]; and mono-phasic
173 dependence (only activation) was found in [64-65].
174 To date, there are two hypothetical mechanism that describe radiation hormesis: this
175 phenomenon is associated with either DNA damage or membrane processes [52,55-56,66-67].
176 Original approach was applied in [64] to test involvement of genetic changes to activation of the
177 bacterial bioluminescence by tritium: using tritium labeled films as a solid source of tritium
178 radiation, the authors demonstrated that tritium activates bacterial luminescence without
179 penetration to the cells. Additionally, no mutations were found in bacterial DNA (16S rRNA gene
180 responsible for vital functions of bacterial cells [68] under the low-intensity irradiation of alpha,
181 beta, and gamma type [65]. A conclusion was made that bioluminescence response is not associated
182 with mutations in the tested gene. Alternative mechanism of biological regulation should be
183 considered, which is related to cell membrane processes, water media ionization, and formation of
184 Reactive Oxygen Species (ROS) under low-dose radiation exposures. Recent results [69]
185 demonstrated that the exposure of marine bacteria to low-intensity irradiation of tritium increases
186 ROS content in the bacterial environment considerably; and a rise of the ROS content correlates with
187 intensification of bacterial bioluminescence intensity. These correlations were explained with
188 "trigger" function of products of tritium decay, signaling role of ROS, and "bystander effect" in the
189 bacterial suspension.
190 Previously [62], effect of americium-241 on luminous bacterium was attributed to ROS
191 generated in aqueous solutions as secondary products of the radioactive decay. The effects of
192 americium-241 and tritium on luminous bacteria were compared in [63] at comparable radiation
193 doses, higher impact of alpha-irradiation of americium-241 was found. The result was related with
194 different energy of radioactive decay of americium-241 and tritium (5637.8 and 18.6 keV [70],
195 respectively) and much higher ROS concentration in americium-241 water solutions as compared to
196 tritiated water. The authors discuss [62] a biological role of ROS generated in aqueous solutions at
197 low-dose exposures.
198 Diffuse reflectance fourier-transform infrared (FTIR) spectroscopic studies [71] showed that the
199 alpha-radioactivity effect of americium-241 "to be transmitted by live cells mainly to the bacterial
200 bioluminescence enzyme system, with negligible structural or compositional changes in cellular
201 macrocomponents."
202 For the first time, bioluminescence of bacteria was used to monitor toxicity of gamma-rays in
203 [72]; effects of average and high doses were under investigation. Exposure of luminous bacteria to

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 August 2019 doi:10.20944/preprints201908.0221.v1

low-dose (≤ 250 mGy) gamma radiation was studied in [61]. Dose-effect dependencies were of stochastic character here, however, the dependencies: exposure time-effect were evident (Figure 8A). Bioluminescence activation was not found under low-dose gamma-radiation exposure; the bioluminescence kinetics corresponded threshold model (Figure 8A), which is supposed as a particular case of hormesis model [2,5]. Probably, lower ionization ability of gamma rays (as compared with alpha and beta particles) is responsible for reducing the bacterial adaptive response.

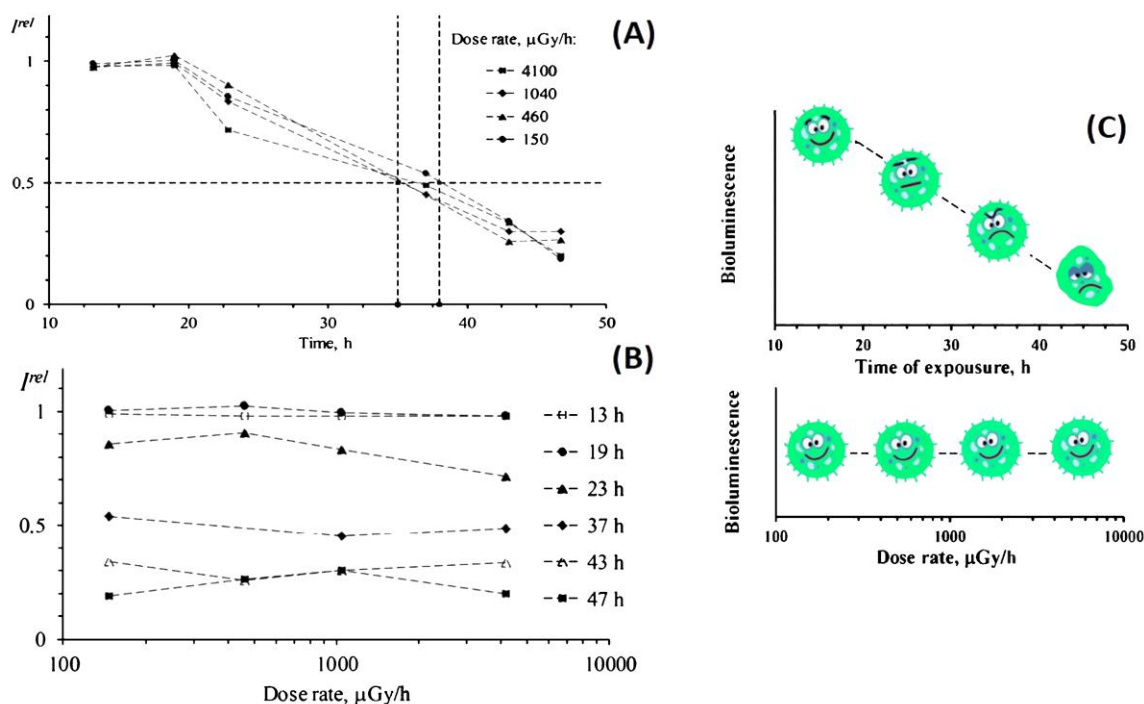


Figure 8. Bioluminescence intensity (I^{rel}) of *P. phosohoreum* exposed to gamma radiation, ^{137}Cs, 20ºC. An error for I^{rel} was 10% [61]. (A) Bioluminescence intensity vs. exposure time; (B) bioluminescence intensity vs. dose rate; (C) schematic presentation of the dependence of bioluminescence intensity on exposure time and dose rate.

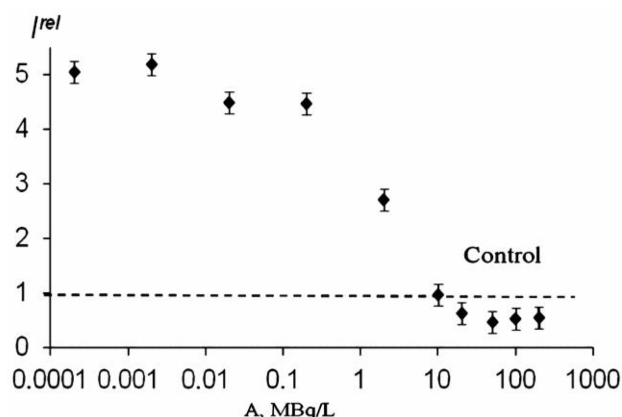
Independence on dose rate of the gamma irradiation was found at the experimental conditions, Figure 8B, similar to low-dose effect of tritium (Figure 7B). The schematic dependency of bioluminescence intensity on exposure time and dose rate is presented in Figure 8C.

One more finding for gamma radiation effects was demonstrated in [61]: lowering temperature (from 20ºC down to 10ºC and 5ºC) decreased sensitivity of the bacteria cells to low-dose gamma-radiation; bioluminescence inhibition was not observed at 10ºC and 5ºC. This result was generally explained by the temperature dependence of metabolic processes including radiation-induced ones.

Hence, experiments with bacterial cells demonstrated independence of their bioluminescence response on intensity of irradiation (activity concentration for alpha/beta radionuclides americium-241/tritium, and dose rate for gamma radiation) under low-dose exposures; however, time dependence was evident corresponding to hormesis (for alpha/beta radionuclides americium-241/tritium) or threshold (for gamma radiation) models. Independency of the response on irradiation intensity (1) and hormesis/threshold type of response vs. time (2) can be considered as a basis for “cellular adaptive response”.

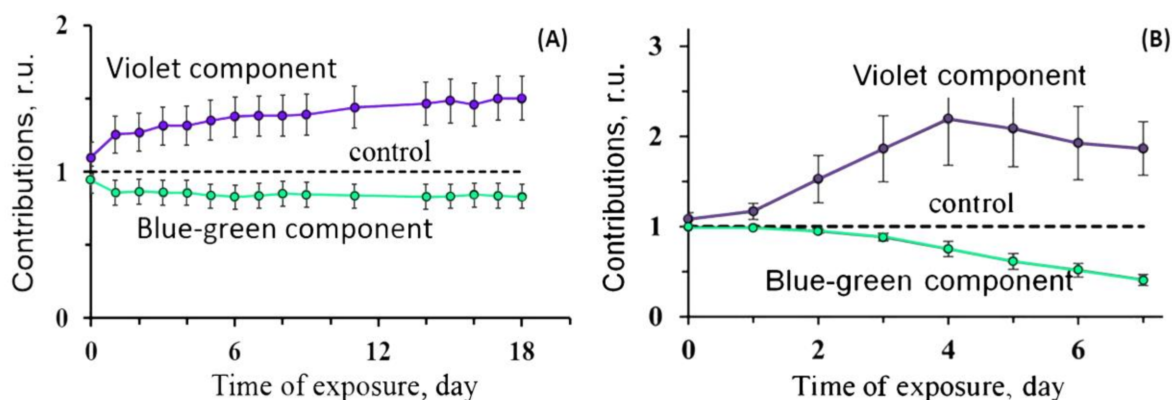
The question is whether these two peculiarities are inherent in a biological system of lower level of organization – enzymatic reactions. Effects of alpha- and beta- emitting radionuclides (americium-241 and tritium) on bioluminescence system of coupled enzyme reactions catalyzed by bacterial luciferase and NADH:FMN-oxidoreductase were studied in [15-16]. Bioluminescence activation and inhibition were observed. Monotonic dependence on concentration of radionuclides was found, too. An example of this dependence is shown in Figure 9.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 August 2019 doi:10.20944/preprints201908.0221.v1



235 **Figure 9.** Bioluminescent intensity of enzyme system, I^{rel}, vs. specific radioactivity of tritiated
236 water, A [16].

237 Paper [63] compared rates of redox processes exposed to americium-241 and tritium: the
238 NADH auto-oxidation rates were much higher in highly-diluted americium-241 solutions, thus
239 contributing to inhibition of NADH-dependent enzymatic processes. This result was compared to
240 ROS concentration in these solutions; a conclusion was made about ROS involvement to the redox
241 transformations of biological low-weigh molecules under the low-intensity radiation exposures.
242 The simplest luminescent bioassay based on CLM-containing protein ("discharged obelin"
243 from *Obelia longissima*) did not demonstrate even activation stage under low-intensity exposures.
244 Figure 10 presents time-courses of colored fluorescence contributions under exposure to beta (A)
245 and gamma (B) irradiation according to [73-74]. The increase of contributions of violet component in
246 both cases is an evidence of proton transfer inhibition in the excited CLM-apoprotein complex, due
247 to the destructive effect of the low-intensity radiation (see Section 2).



248 **Figure 10.** Relative contributions of spectral components to fluorescence spectra of CLM-containing
249 protein ("discharged obelin" from *Obelia longissima)* exposed to (A) tritiated water, 200 MBq/L [73];
250 (B) gamma radiation, ^{137}Cs, 2 mGy/h, 20°C [74].

251 Dependence of the response of CLM-containing protein to low-intensive gamma radiation on
252 temperature was demonstrated in [74] as well.
253 Hence, simplest bioassay systems (based on enzymatic reaction and physicochemical processes
254 in protein complex) show lower ability for adaptive response to low-dose radioactive exposures. In
255 contrast to bacterial cells, they can demonstrate dependence on intensity of irradiation or absence of
256 the activation stage in the course of chronic exposure. However, a lack of experimental results does
257 not allow making definite conclusions. The study should be developed in this direction.

258 4. Low-concentration effects of bioactive compounds: antioxidant activity via bioluminescence 259 bioassays

Investigation of low-concentration effects of bioactive compounds has been developed from 60th of the previous century [52,75-77] Hormesis concept progresses to describe intensification of physiological functions of organisms under low-concentration exposures [2-4,76-79].

Bioluminescence bioassays based on luminous marine bacteria and their enzymes are excellent tools for study low-concentration effects due to their features mentioned before:

1. high rates of test procedure which
    - provide statistical reliability of the bioassay results and
    - exclude genetic level of analysis and appeal to biochemical, chemical and physicochemical processes in cells;
2. possibility to compare effects of bioactive compounds at different organization levels – cellular and enzymatic.

The bioassays based on luminous marine bacterium and bacterial enzymes were used to study antioxidant properties of the bioactive compounds of natural and artificial origination. Humic substances and fullerenols were chosen as examples of these compounds, Figure 11. Humic substances are products of natural oxidative transformation of organic matter in soils and sediments, attenuators of toxicity in natural water bodies and soils. Fullerenols are specific allotropic form of carbon, nanosized polyhydroxylated water-soluble derivatives of fullerenes, bioactive compounds, and perspective pharmaceutical agents. Hypothetical structures of humic substances and fullerenol are presented in Figure 11. Antioxidant activity of humic substances was studied in [80-84] and fullerenols – in [84-87].

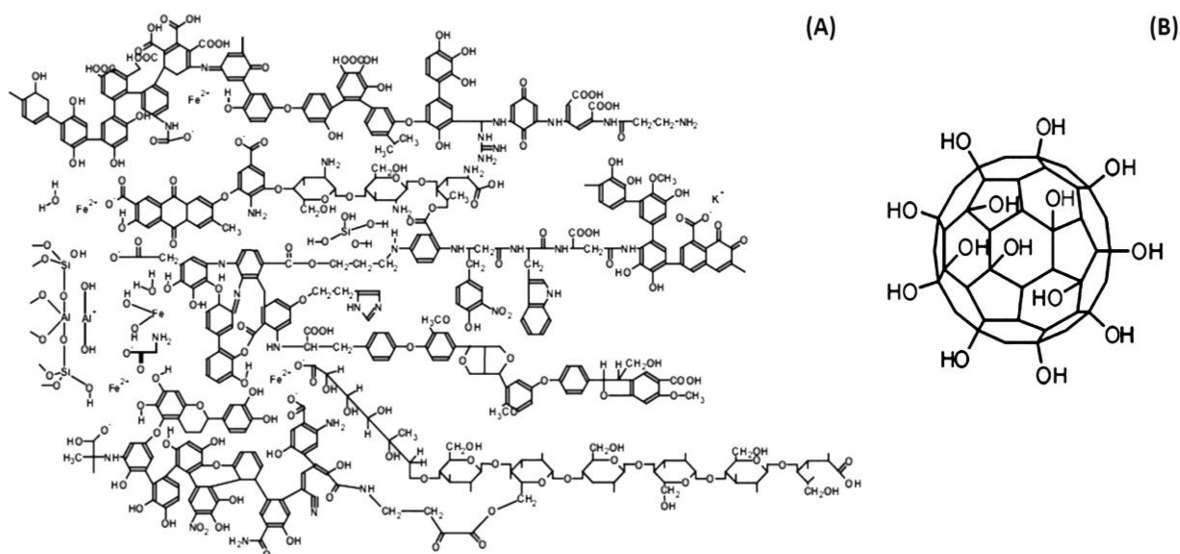


Figure 11. Hypothetical structure of bioactive compounds: A –fragment of humic substances [88], B – fullerenol C-60.

Cellular and enzymatic assays were used to evaluate a General Toxicity (GT) under conditions of the oxidative stress; this stress was modeled in the solutions of exogenous organic or inorganic oxidizers (1,4-benzoquinone or potassium ferricianide, respectively). Additionally, enzymatic bioassay was shown to be specific to oxidizers [25,89]; it can be used to monitor an oxidative toxicity (OxT) of the solutions. This toxicity type characterizes the redox properties of toxic compounds, while the other type of toxicity, general toxicity (GT) mentioned before, integrates all the interactions of toxic compounds with bioassay system: redox reactions, non-polar and polar interactions, so on. The GT is concerned with suppression of maximal bioluminescence intensity, while the OxT uses a specific kinetic parameter, induction period of bioluminescence. The latter appears in the presence of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 August 2019 doi:10.20944/preprints201908.0221.v1

exogenous oxidizers, depending on their redox potential and concentration [25]. Justification for GT and OxT application to evaluate toxicity of bioactive compounds is presented in [80-87].

Principle of antioxidant efficiency evaluation is presented in Figure 12 for bacteria-based (upper line) and enzyme-based (lower line) bioassays; humic substances are chosen here as an example of an antioxidant agent.

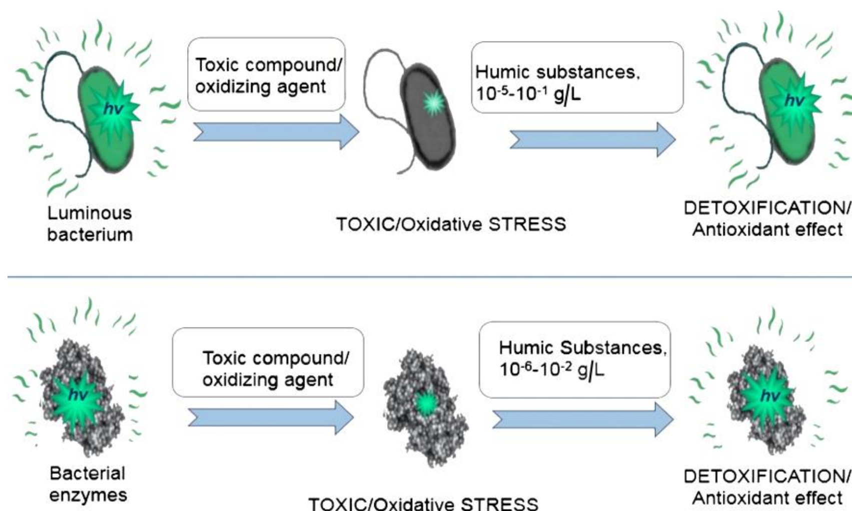


Figure 12. Principle of antioxidant efficiency evaluation using bacteria-based (upper line) and enzyme-based (lower line) bioluminescent assays.

Antioxidant efficiency of bioactive compounds was characterized by antioxidant coefficients D_{GT} or D_{OxT}, corresponding to GT or OxT monitoring. Values of D_{GT} >1 or D_{OxT} >1 revealed antioxidant effects of the bioactive compounds.

Paper [86] compares biological activity of carbon nano-structures of natural and artificial origination, i.e. humic substances and fullerenols. The representative of the group of fullerenols, $C_{60}O_y(OH)_x$ where y+x =20–22, was chosen. Both bioassays were used to monitor toxicity and antioxidant activity of the bioactive compounds. Toxic concentrations of humic substances and fullerenol, inhibiting bioluminescence of the assay systems were determined and excluded from further antioxidant experiments. Antioxidant coefficients of the bioactive compounds and ranges of their active concentrations were determined in solutions of model oxidizers. Figure 13 presents an example of dependencies of antioxidant coefficients D_{OxT} on concentration of fullerenol, *F* (A) and humic substances, *HS* (B).

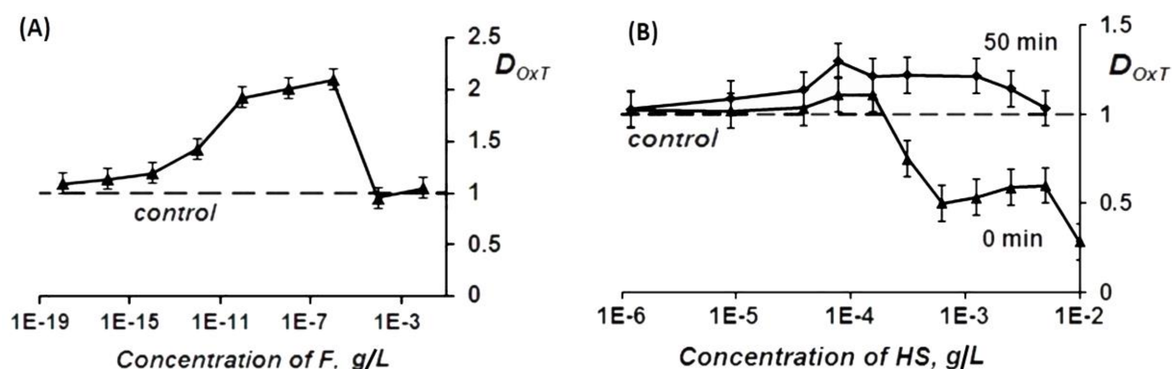


Figure 13. Antioxidant coefficients D_{OxT} of fullerenol, *F* (A), and humic substances, *HS* (B) in model solution of organic oxidizer (1,4-benziquinone) [86]. Time of incubation of humic substances with oxidizer (0 min and 50 min) is indicated in Figure (B). Enzymatic assay.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 August 2019 doi:10.20944/preprints201908.0221.v1

313 Both *HS* and *F* demonstrated low-concentration antioxidant activity (D_{OxT} >1); however,
314 quantitative antioxidant characteristics were different: fullerenol′ D_{OxT} -values were higher, its
315 antioxidant activity covered wider concentration range as seen from Figure 13. Antioxidant activity
316 of *HS* was found to be time-dependent, Figure 13(B), while the *F*′ antioxidant effect showed
317 independency on time. The *HS*′ antioxidant effect did not depend on amphiphilic characteristics of
318 the environment (D_{OxT} -values were 1.3 in the solutions of inorganic and organic oxidizers), while
319 D_{OxT} of *F* was maximal in solutions organic oxidizer (D_{OxT} = 2.0). The difference in effects of bioactive
320 compounds in solutions of organic and inorganic oxidizers can be concerned with their
321 hydrophobic interactions in enzymes or cellular membranes. Changes in fluidity and structural
322 organization of lipid bilayers in hydrophobic fragments of membranes by fullerenol F-60 were
323 previously reported in [90].
324 In [86], the differences in toxic and antioxidant effects of fullerenol and humic substances were
325 attributed to the structure of these compounds. Non-rigidity of humic macromolecules determines
326 their diffusion restrictions, which result in higher toxicity and time-dependence of their antioxidant
327 coefficients. The ability to decrease ROS content in water solutions probably contributes to higher
328 toxicity of humates, as well. Non-rigidity and polyfunctionality can be responsible for unification of
329 humate′ properties in solutions of oxidizers of different hydrophilic/hydrophobic characteristics.
330 Low-concentration antioxidant activity was explained by catalytic redox activity of π-fragments of
331 the bioactive structures.
332 Antioxidant property of highly diluted solutions of fullerenols was attributed [85] to hormesis
333 phenomenon. The bacteria-based and enzyme-based assays demonstrated similar peculiarities of the
334 antioxidant processes: “(1) ultralow concentrations of fullerenols were active (*ca* 10^{-17}–10^{-4} and
335 10^{-17}–10^{-5} g/L, respectively), (2) no monotonic dependence of detoxification efficiency on fullerenol
336 concentrations was observed, and (3) detoxification of organic oxidizer solutions was more effective
337 than that of the inorganic oxidizer”. The antioxidant properties were concerned with adaptive
338 cellular response under low-dose exposures. The sequence analysis of 16S ribosomal RNA was
339 carried out for long-term exposures; mutations in bacterial DNA were not revealed. The conclusion
340 was suggested that hydrophobic interactions might be involved to the antioxidant mechanism.
341 Biological efficiency of low and ultralow concentrations of hydrated fullerenes was studied
342 and discussed earlier in [91-92]. This effect was attributed to the fullerene′ ability to regulate
343 dynamic structure of aqueous media and to adjust “redox processes (especially those involving
344 oxygen) in aqueous systems”. Earlier [93], a role of aqueous media in fullerenol′ antiradical activity
345 was discussed. Probably, ROS in aqueous media are able to contribute to the antioxidant effect of
346 fullerenols.
347 In [84,87] the antioxidant activity of fullerenols was related with modification of their surface by
348 oxygen substituents, as well as presence of exo- or endo-hedral metal atoms. Difference in the
349 antioxidant activity of these fullerenols was explained through their electron donor/acceptor
350 properties and different catalytic activity.
351 Paper [81] studied combined influence of humic substances (HS) and salts of aliovalent metals
352 on the bioluminescent assay systems, the rates of biochemical reactions and bacterial ultrastructure
353 were analyzed. The detoxifying/antioxidant effects of HS were explained [81-83] by: (1) “a decrease
354 of free metal content in water solutions under metal–HS binding; (2) increase of biochemical reaction
355 rates in a bioluminescent assay system under HS effect; (3) enhancement of mucous layers on cell
356 surface as a response to unfavorable impact of toxicants. Detoxifying mechanisms (2) and (3) reveal
357 the active role of bioassay systems in detoxification processes”.
358 As an outlook:
359 1. Bioactive compounds can produce toxic (inhibiting) and antioxidant effects. Toxic effect of
360 humic substances was found at higher concentrations (>0.1 g/L for bacterial assay and >0.01 g/L for
361 enzymatic assay), an antioxidant effect was found at lower concentrations. All fullerenols showed
362 toxic effects at higher concentrations: from >0.01 to >0.001 g/L; their antioxidant effect was found at
363 low and ultralow concentrations: *ca* 10^{-17}-10^{-4} and 10^{-17}-10^{-5} g/L for bacteria-based and enzyme-based

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 August 2019 doi:10.20944/preprints201908.0221.v1

assays, respectively. The range of active fullerenol' concentrations might correspond to several tens of molecules per liter.

2. No monotonic dependencies of antioxidant coefficients on concentration of bioactive compounds were found in a wide concentration range (up to 15-17 orders for fullerenols).

3. Both bacterial and enzymatic assays demonstrated antioxidant effect of bioactive compounds. This result reveals role of biochemical and physicochemical processes in low-concentration antioxidant effects of bioactive compounds.

4. Cellular and enzymatic bioluminescent assays showed that detoxification of solutions of organic oxidizers was more effective than inorganic oxidizers, with this indicating the importance of hydrophobic interactions in the antioxidant mechanism.

Hence, similar to low-dose radiation effects, antioxidant effects of bioactive compounds (fullerenols and humic substances) showed (a) positive response of the luminous bacteria and their enzymes to low-concentration exposure and (b) absence of linear concentration-effect dependencies.

5. Conclusions

Luminescence bioassay systems can provide non-genetic approach to low-intensity exposures due to high rate of registration of luminescence response. Possibility to study biochemical, chemical and physicochemical (polar, apolar, hydrophobic) processes using luminescence signaling is a contribution to exposomic concept [28-31].

As we assume, the biochemical, chemical and physicochemical processes have not attract, until now, much attention in frames of hormesis conception. However, compensatory effects can be strictly studied in terms of chemical equilibrium, by analyzing the individual equilibrium constants in complex systems of coupled chemical reactions. The compensatory effects can be considered, in this case, as a result of equilibrium disturbing followed by concentrational reconstruction of the whole system, streaming to the new equilibrium with the new equilibrium concentrations. While streaming to the new balance, fluctuations of concentrations are possible, as well as involvement of new processes to the system of coupled reactions, with this providing "over-compensative" response to the exposures.

The results presented in this review reveal the dependences of the biological response on time of exposure to low-intensity factors, as well its independencies on radioactivity or concentration. Uncertainty of dose-response relations was paid attention, too. Differences in dependencies of luminescence responses on time and exposure intensity should be analyzed in detail at molecular level in further experiments.

Funding: This work was supported by PRAN-32, Program: "Nanostructures: physics, chemistry, biology, technological basis"; RFBR N 18-29-19003; RFBR-Krasnoyarsk Regional Foundation N 18-44-240004, 18-44-242002.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the writing of the manuscript, or in the decision to publish the results.

Abbreviations

CLM	Coelenteramide
F	Fullerenol
FTIR	Fourier-transform infrared
GT	General Toxicity
HS	Humic Substances
NADH	Nicotinamide adenine dinucleotide
OxT	Oxidative Toxicity
ROS	Reactive oxygen species

References

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 August 2019 doi:10.20944/preprints201908.0221.v1

1. Southam, C.M.; Ehrlich, J. Effects of extracts of western red-cedar heartwood on certain wood-decaying fungi in culture. *Phytopathology* **1943**, *33*, 517-524.
2. Calabrese, E.J. Hormesis: Path and Progression to Significance. *Int. J. Mol. Sci.* **2018**, *19*, 2871-2886, doi:10.3390/ijms19102871.
3. Calabrese, E.J. Hormetic mechanisms. *Crit. Rev. Toxicol*. **2013**, *43*, 580–606, doi:10.3109/10408444.2013.808172.
4. Calabrese, E.J. Hormesis: a fundamental concept in biology. *Microb. Cell* **2014**, *1*, 145-149, doi:10.15698/mic2014.05.145.
5. Iavicoli, I.; Leso, V.; Fontana, L.; Calabrese, E.J. Nanoparticle exposure and hormetic dose–responses: An update. *Int. J. Mol. Sci.* **2018**, *19*, 805, doi:10.3390/ijms19030805.
6. Vasilenko, I.Ya.; Vasilenko, O.I. Radiation risk when exposed to small doses is negligible. *Atomic Energy Bulletin* **2001**, *12*, 34-37 (In Russian).
7. Bulich, A.A.; Isenberg, D.L. Use of the luminescent bacterial system for rapid assessment of aquatic toxicity. *ISA Trans.***1981**, *20*, 29–33.
8. Abbas, M.; Adil, M.; Ehtisham-Ul-Haque, S.; Munir, B.; Yameen, M.; Ghaffar, A.; Shar, G.A.; Asif Tahir, M.; Iqbal, M. Vibrio fischeri bioluminescence inhibition assay for ecotoxicity assessment: A review. *Sci. Total Environ.* **2018,** *626*, 1295–1309, doi:10.1016/j.scitotenv.2018.01.066.
9. Fedorova, E.; Kudryasheva, N.; Kuznetsov, A.; Mogil'naya, O.; Stom, D. Bioluminescent monitoring of detoxification processes: Activity of humic substances in quinone solutions. *J. Photochem. Photobiol. B* **2007**, *88*, 131–136, doi:10.1016/j.jphotobiol.2007.05.007.
10. Girotti, S.; Ferri, E.N.; Fumo, M.G.; Maiolini, E. Monitoring of environmental pollutants by bioluminescent bacteria. *Anal. Chim. Acta* **2008**, *608*, 2–29, doi:10.1016/j.aca.2007.12.008.
11. Kudryasheva, N.; Kratasyuk, V.; Esimbekova, E.; Vetrova, E.; Nemtseva, E.; Kudinova, I. Development of bioluminescent bioindicators for analyses of environmental pollution. *Field Anal. Chem. Tech.* **1998**, *2*, 277–280, doi:10.1002/(SICI)1520-65212:5<277::AID-FACT4>3.0.CO;2-P.
12. Roda, A.; Pasini, P.; Mirasoni, M.; Michchelini, E.; Guardigli, M. Biotechnological application of bioluminescence and chemiluminescence. *Trends Biotech.* **2004**, *22*, 295–303, doi:10.1016/j.tibtech.2004.03.011.
13. Kudryasheva, N.S.; Rozhko, T.V. Effect of low-dose ionizing radiation on luminous marine bacteria: radiation hormesis and toxicity. *J. Environ. Radioact*. **2015**, *142*, 68-77, doi:10.1016/j.jenvrad.2015.01.012.
14. Kratasyuk, V.A.; Esimbekova, E.N. Applications of luminous bacteria enzymes in toxicology. *Comb. Chem. High Throughput Screen.* **2015**, *18*, 952–959, doi:10.2174/1386207318666150917100257.
15. Rozhko, T.V.; Kudryasheva, N.S.; Kuznetsov, A.M.; Vydryakova, G.A.; Bondareva, L.G.; Bolsunovsky, A.Ya. Effect of low-level α-radiation on bioluminescent assay systems of various complexity. *Photochem. Photobiol. Sci.* **2007**, *6*, 67–70, doi:10.1039/B614162P.
16. Selivanova, M.A.; Mogilnaya, O.A.; Badun, G.A.; Vydryakova, G.A.; Kuznetsov, A.M.; Kudryasheva, N.S. Effect of tritium on luminous marine bacteria and enzyme reactions. *J. Environ. Radioact*. **2013**, *120*, 19–25, doi:10.1016/j.jenvrad.2013.01.003.
17. Esimbekova, E.N.; Kondik, A.M.; Kratasyuk, V.A. Bioluminescent enzymatic rapid assay of water integral toxicity. *Environ. Monit. Assess*. **2013**, *185*, 5909–5916, doi:10.1007/s10661-012-2994-1.
18. Efremenko, E.N.; Maslova, O.V.; Kholstov, A.V.; Senko, O.V.; Ismailov, A.D. Biosensitive element in the form of immobilized luminescent photobacteria for detecting ecotoxicants in aqueous flow-through systems. *Luminescence* **2016**, *31*, 1283-1289, doi:10.1002/bio.3104.
19. Ismailov, A.D.; Aleskerova, L.E. Photobiosensors containing luminescent bacteria. *Biochem.* **2015**, *80*, 733–744, doi:10.1134/S0006297915060085.
20. Ranjan, R.; Rastogi, N.K.; Thakur, M.S. Development of immobilized biophotonic beads consisting of Photobacterium leiognathi for the detection of heavy metals and pesticide. *J. Hazard Mater.* **2012**, *225–226*, 114–123, doi:10.1016/j.hazmat.2012.04.076.
21. Kudryasheva, N.S. Bioluminescence and exogenous compounds: Physicochemical basis for bioluminescence assay. *J. Photochem. Photobiol. B* **2006**, *83*, 77–86, doi:10.1016/j.jphotobiol.2005.10.003.
22. Kirillova, T.N.; Kudryasheva, N.S. Effect of heavy atom in bioluminescent reactions. *Anal. Bioanal. Chem.* **2007**, *387*, 2009–2016, doi:10.1007/s00216-006-1085-y.

456 23. Kirillova, T.N.; Gerasimova, M.A.; Nemtseva, E.V.; Kudryasheva, N.S. Effect of halogenated fluorescent
457 compounds on bioluminescent reactions. *Anal. Bioanal. Chem.* **2011**, *400*, 343–351,
458 doi:10.1007/s00216-011-4716-x.
459 24. Nemtseva, E.V.; Kudryasheva, N.S. The mechanism of electronic excitation in bacterial bioluminescent
460 reaction. *Russ. Chem. Rev.* **2007**, *76*, 91–100, doi:10.1070/RC2007v076n01ABEH003648.
461 25. Vetrova, E.V.; Kudryasheva, N.S.; Kratasyuk, V.A. Redox compounds influence on the
462 NAD(P)H:FMN-oxidoreductase-luciferase bioluminescent system. *Photochem. Photobiol. Sci.* **2007**, *6*, 35–40,
463 doi:10.1039/b608152e.
464 26. Rappaport, S.M.; Smith, M.T. Epidemiology. Environment and disease risks. *Science* **2010**, *330*, 460–461,
465 doi:10.1126/science.1192603.
466 27. Wild, C.P. The exposome: from concept to utility. *Int. J. Epidemiol.* **2012**, *41*, 24–32, doi:10.1093/ije/dyr236.
467 28. Siroux, V.; Agier, L.; Slama, R. The exposome concept: a challenge and a potential driver for
468 environmental health research. *Eur. Respir. Rev.* **2016**, *25*, 124-129, doi:10.1183/16000617.0034-2016.
469 29. Wild, C.P. Complementing the Genome with an "Exposome": The Outstanding Challenge of
470 Environmental Exposure Measurement in Molecular Epidemiology. *Cancer Epidemiol. Biomark. Prev.* **2005**,
471 *14*, 1847–1850, doi:10.1158/1055-9965.EPI-05-0456.
472 30. Zubova, N.N.; Bulavina, A.Y., Savitsky, A.P. Spectral and physicochemical properties of green (GFP) and
473 red (drFP583) fluorescent proteins. *Usp. Biol. Khim.* **2003**, *43*, 163–224 (In Russian).
474 31. Stepanenko, O.V.; Verkhusha, V.V.; Kuznetsova, I.M.; Turoverov, K.K. Fluorescent proteins:
475 physical-chemical properties and application in cell biology. *TSITOLOGIYA* **2007**, *49*, 395-420.
476 32. Kumar, A.; Pal. D. Green fluorescent protein and their applications in advance research. *Res. J. Appl. Sci.*
477 *Eng. Tech.* **2016**, *1*, 42-46.
478 33. Remington, S.J. Green fluorescent protein: a perspective. *Protein Sci.* **2011**, *20*, 1509–1519,
479 doi:10.1002/pro.684.
480 34. Frank, L.A. Ca^{2+}-Regulated photoproteins: effective immunoassay reporters. *Sensors* **2010**, *10*, 11287–1130,
481 doi:10.3390/s101211287.
482 35. Krasitskaya, V.V.; Burakova, L.P.; Pyshnaya, I.A.; Frank, L.A. Bioluminescent reporters for identification
483 of gene allelic variants. *Russian Journal of Bioorganic Chemistry* **2012**, *38*, 298–305,
484 doi:10.1134/S1068162012030090.
485 36. Shimomura, O.; Teranishi, K. Light-emitters involved in the luminescence of coelenterazine. *Luminescence*
486 **2000**, *15*, 51-58, doi:10.1002/(SICI)1522-7243(200001/02)15:1<51::AID-BIO555>3.0.CO;2-J.
487 37. Li, Z.-S.; Zhao, X.; Zou, L.-Y.; Ren, A.-M. The Dynamics Simulation and Quantum Calculation
488 Investigation. About Luminescence Mechanism of Coelenteramide. *Photochem. Photobiol.* **2013**, *89*, 849–855,
489 doi:10.1111/php.12073.
490 38. Malikova, N.P.; Stepanyuk, G.A.; Frank, L.A.; Markova, S.V.; Vysotski, E.S.; Lee, J. Spectral tuning of
491 obelin bioluminescence by mutations of Trp92. *FEBS Letters* **2003**, *554*, 184-188,
492 doi:10.1016/S0014-5793(03)01166-9.
493 39. Belogurova, N.V.; Kudryasheva, N.S.; Alieva, R.R. Activity of upper electron-excited states in
494 bioluminescence of coelenterates. *J. Mol. Struct.* **2009**, *924*, 148–152, doi:10.1016/j.molstruc.2008.11.014.
495 40. Sharifian, S.; Homaei, A.; Hemmati, R.; B. Luwor, R.; Khajeh, K. The emerging use of bioluminescence in
496 medical research. *Biomed. Pharmacother.* **2018**, *101*, 74-86, doi:10.1016/j.biopha.2018.02.065.
497 41. Lee, J. Perspectives on Bioluminescence Mechanisms. *Photochem. Photobiol.* **2017**, *93*, 389–404,
498 doi:10.1111/php.12650.
499 42. Chen, S.-F.; Ferre, N.; Liu, Y.-J. QM/MM Study on the Light Emitters of Aequorin Chemiluminescence,
500 Bioluminescence, and Fluorescence: A General Understanding of the Bioluminescence of Several Marine
501 Organisms. *Chem. Eur. J.* **2013**, *19*, 8466–8472, doi:10.1002/chem.201300678.
502 43. Alieva, R.R.; Tomilin, F.N.; Kuzubov, A.A.; Ovchinnikov, S.G.; Kudryasheva, N.S. Ultraviolet
503 fluorescence of coelenteramide and coelenteramide-containing fluorescent proteins. Experimental and
504 theoretical study. *J. Photochem. Photobiol. B* **2016**, *162*, 318-323, doi:10.1016/j.jphotobiol.2016.07.004.
505 44. Min, C.-G.; Li, Z.-S.; Ren, A.-M; Zou, L.-Y; Guo, J.-F.; Goddard, J.D. The fluorescent properties of
506 coelenteramide, a substrate of aequorin and obelin. *J. Photochem. Photobiol. A* **2013**, *251*, 182–188,
507 doi:10.1016/j.jphotochem.2012.10.028.
508 45. van Oort, B.; Eremeeva, E.V.; Koehorst, R.B.M.; Laptenok, S.P.; van Amerongen, H.; van Berkel, W.J.H.;
509 Malikova, N.P.; Markova, S.V.; Vysotski, E.S.; Visser, A.J.W.G.; Lee, J. Picosecond Fluorescence Relaxation

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 August 2019 doi:10.20944/preprints201908.0221.v1

510 Spectroscopy of the Calcium-Discharged Photoproteins Aequorin and Obelin. *Biochem.* **2009**, *48*,
511 10486–10491, doi:10.1021/bi901436m.
512 46. Belogurova, N.V.; Kudryasheva, N.S.; Alieva, R.R.; Sizykh, A.G. Spectral components of bioluminescence
513 of aequorin and obelin. *J. Photochem. Photobiol. B* **2008**, *92*, 117-122, doi:10.1016/j.jphotobiol.2008.05.006.
514 47. Belogurova, N.V.; Kudryasheva, N.S. Discharged photoprotein Obelin: Fluorescence peculiarities. *J.*
515 *Photochem. Photobiol. B* **2010**, *101*, 103–108, doi:10.1016/j.jphotobiol.2010.07.001.
516 48. Alieva, R.R.; Belogurova, N.V.; Petrova, A.S.; Kudryasheva N.S. Fluorescence properties of
517 Ca^{2+}-independent discharged obelin and its application prospects. *Anal. Bioanal. Chem.* **2013**, *405*,
518 3351-3358, doi:10.1007/s00216-013-6757-9.
519 49. Gao, M.; Liu, Y.-J. Photoluminescence Rainbow from Coelenteramide—A Theoretical Study. *Photochem.*
520 *Photobiol*. **2018**, *95*, 563-571, doi:10.1111/php.12987.
521 50. Alieva, R.R.; Kudryasheva, N.S. Variability of fluorescence spectra of coelenteramide-containing proteins
522 as a basis for toxicity monitoring. *Talanta* **2017**, *170*, 425-431, doi:10.1016/j.talanta.2017.04.043.
523 51. Alieva, R.R.; Belogurova, N.V.; Petrova, A.S.; Kudryasheva, N.S. Effects of alcohols on fluorescence
524 intensity and color of a discharged-obelin-based biomarker. *Anal. Bioanal. Chem*. **2014**, *406*, 2965-2974,
525 doi:10.1007/s00216-014-7685-z.
526 52. Burlakova, E.B.; Konradov, A.A.; Maltseva, E.X. Effect of extremely weak chemical and physical stimuli on
527 biological systems. *Biophysics (Moscow)* **2004**, *49*, 522-534.
528 53. Feinendegen, L.E.; Pollycove, M.; Neumann, R.D. Whole-body responses to low-level radiation exposure:
529 New concepts in mammalian radiobiology. *Exp. Hematol.* **2007**, *35*, 37-46, doi:10.1016/j.exphem.2007.01.011.
530 54. Feinendegen, L.E. Evidence for beneficial low level radiation effects and radiation hormesis. *Br. J. Radiol.*
531 **2005**, *78*, 3-7, doi:10.1259/bjr/63353075.
532 55. Mothersill, C.; Seymour, C. Implications for human and environmental health of low doses of ionising
533 radiation. *J. Environ. Radioact.* **2014**, *133*, 5-9, doi:10.1016/j.jenvrad.2013.04.002.
534 56. Jo, E.-R.; Jung, P.-M.; Choi, J.; Lee, J.-W. Radiation sensitivity of bacteria and virus in porcine xenoskin for
535 dressing agent. *Radiat. Phys. Chem.* **2012**, *81*, 1259-1262, doi:10.1016/j.radphyschem.2011.08.016.
536 57. Mesquita, N.; Portugal, A.; Piñar, G.; Loureiro, J.; Coutinho, A.P.; Trovão, J.; Nunes, I.; Botelho, M.L.;
537 Freitas, H. Flow cytometry as a tool to assess the effects of gamma radiation on the viability, growth and
538 metabolic activity of fungal spores. *Int. Biodeter. Biodegr*. **2013**, *84*, 250-257, doi:10.1016/j.ibiod.2012.05.008.
539 58. Paul, J.; Kadam, A.A.; Govindwar, S.P.; Kumar, P.; Varshney, L. An insight into the influence of low dose
540 irradiation pretreatment on the microbial decolouration and degradation of Reactive Red-120 dye.
541 *Chemosphere* **2013**, *90*, 1348-1358, doi:10.1016/j.chemosphere.2012.07.049.
542 59. Xavier, M.P.; Dauber, C.; Mussio, P.; Delgado, E.; Maquieira, A.; Soria, A.; Curuchet, A.; Márquez, R.;
543 Méndez, C.; López, T. Use of mild irradiation doses to control pathogenic bacteria on meat trimmings for
544 production of patties aiming at provoking minimal changes in quality attributes. *Meat Sci.* **2014**, *98*,
545 383-391, doi:10.1016/j.meatsci.2014.06.037.
546 60. Luckey, T.D. *Hormesis with Ionizing Radiation*; Publisher: CRC Press, Incorporated Boca Raton, Florida,
547 1980; 225 pp.
548 61. Kudryasheva, N.S.; Petrova, A.S.; Dementyev, D.V.; Bondar, A.A. Exposure of luminous marine bacteria
549 to low-dose gamma-radiation. *J. Environ. Radioact.* **2017**, *169-170*, 64-69, doi: 10.1016/j.jenvrad.2017.01.002.
550 62. Alexandrova, M.; Rozhko, T.; Vydryakova, G.; Kudryasheva, N. Effect of americium-241 on luminous
551 bacteria. Role of peroxides. *J. Environ. Radioact.* **2011**, *102 (4)*, 407-411, doi:10.1016/j.jenvrad.2011.02.011.
552 63. Selivanova, M.A.; Rozhko, T.V.; Devyatlovskaya, A.N.; Kudryasheva, N.S. Comparison of chronic
553 low-dose effects of alpha-and beta-emitting radionuclides on marine bacteria. *Cent. Eur. J. Biol.* **2014**, *9*,
554 951-959, doi:10.2478/s11535-014-0331-0.
555 64. Rozhko, T.V.; Badun, G.A.; Razzhivina, I.A.; Guseynov, O.A.; Guseynova, V.E.; Kudryasheva, N.S. On
556 mechanism of biological activation by tritium. *J. Environ. Radioact.* **2016**, *157*, 131–135,
557 doi:10.1016/j.jenvrad.2016.03.017.
558 65. Rozhko, T.V.; Guseynov, O.A.; Guseynova, V.E.; Bondar, A.A.; Devyatlovskaya, A.N.; Kudryasheva, N.S.
559 Is bacterial luminescence response to low-dose radiation associated with mutagenicity? *J. Environ.*
560 *Radioact.* **2017**, *177*, 261–265, doi:10.1016/j.jenvrad.2017.07.010.
561 66. Albers, R.W. Biochemical aspects of active transport. *Annu. Rev. Biochem.* **1967**, *36*, 727-756.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 August 2019 doi:10.20944/preprints201908.0221.v1

562 67. Lloyd, D.C.; Edvards, A.A.; Leonard, A.; Deknut, G.L.; Verschaeve, L.; Natarajan, A.T.; Darrudi, F.;
563 Obe, G.; Palitti, F.; Tanzarella, C.; Tawn, E.J. Chromosomal aberrations in human lymphocytes induced in
564 vitro by very low doses of X-rays. *Int. J. Radiat. Biol.* **1992**, *61*, 335-343, doi:10.1080/09553009214551021.
565 68. Clarridge, J.E. Impact of 16S rRNA Gene Sequence Analysis for Identification of Bacteria on Clinical
566 Microbiology and Infectious Diseases. *Clin. Microbiol. Rev.* **2004**, *17*, 840–862,
567 doi:10.1128/CMR.17.4.840-862.2004.
568 69. Rozhko, T.; Nogovitsyna, E.; Badun, G.; Lukyanchuk, A.; Kudryasheva, N. Reactive Oxygen Species and
569 Low-Dose Effects of Tritium on Bacterial Cells. *J. Environ. Radioact., under revision.*
570 70. Audi, G.; Wapstra, A.H.; Thibault, C. The AME2003 atomic mass evaluation (II).Tables, graphs, and
571 references. *Nucl. Phys. A* **2003**, *729*, 337–676, doi:10.1016/j.nuclphysa.2003.11.003.
572 71. Kamnev, A.A.; Tugarova, A.V.; Selivanova, M.A.; Tarantilis, P.A.; Polissiou, M.G.; Kudryasheva, N.S.
573 Effects of americium-241 and humic substances on *Photobacterium phosphoreum*: Bioluminescence and
574 diffuse reflectance FTIR spectroscopic studies. *Spectrochim. Acta A: Mol. Biomol. Spectrosc.* **2013**, *100*,
575 171-175, doi:10.1016/j.saa.2012.06.003.
576 72. Min, J.; Lee, C.W.; Gu, M.B. Gamma-radiation dose-rate effects on DNA damage and toxicity in bacterial
577 cells. *Radiat. Environ. Bioph.* **2003**, *42*, 189-192, doi:10.1007/s00411-003-0205-8.
578 73. Petrova, A.S.; Lukonina, A.A.; Badun, G.A.; Kudryasheva, N.S. Fluorescent Coelenteramide-Containing
579 Protein as a Color Bioindicator for Low-Dose Radiation Effects. *Anal. Bioanal. Chem*. **2017**, *409*, 4377–4381,
580 doi:10.1007/s00216-017-0404-9.
581 74. Petrova, A.S.; Lukonina, A.A.; Dementyev, D.V.; Bolsunovsky, A.Ya.; Popov, A.V.; Kudryasheva, N.S.
582 Protein-based fluorescent bioassay for low-dose gamma radiation exposures. *Anal. Bioanal. Chem*. **2018**,
583 *410*, 6837-6844, doi: 10.1007/s00216-018-1282-5.
584 75. Selye, H. Changing distress into eustress: voices theories on stress. *Tex. Med.* **1980**, *76*, 78-80.
585 76. Wang, C.-R.; Tian, Y.; Wang, X.-R.; Yu, H.-X.; Lu, X.-W.; Wang, C.; Wang, H. Hormesis effects and
586 implicative application in assessment of lead-contaminated soils in roots of *Vicia faba* seedlings.
587 *Chemosphere* **2010**, *80*, 965-971, doi:10.1016/j.chemosphere.2010.05.049.
588 77. Baldwin, J.; Grantham, V. Radiation hormesis: historical and current perspectives. *J. Nucl. Med. Technol.*
589 **2015**, *43*, 242-246, doi:10.2967/jnmt.115.166074.
590 78. Kaiser, J. Hormesis: sipping from a poisoned chalice. *Science* **2003**, *302*, 376–379,
591 doi:10.1126/science.302.5644.376.
592 79. Calabrese, E.J.; Baldwin, L.A. The frequency of U-shaped dose responses in the toxicological literature.
593 *Toxicol. Sci.* **2001**, *62*, 330–338, doi:10.1093/toxsci/62.2.330.
594 80. Tarasova, A.S.; Stom, D.I.; Kudryasheva, N.S. Effect of humic substances on toxicity of inorganic oxidizer
595 bioluminescent monitoring. *Environ. Toxicol. Chem*. **2011**, *30*, 1013–1017, doi:10.1002/etc.472.
596 81. Tarasova, A.S.; Kislan, S.L.; Fedorova, E.S.; Kuznetsov, A.M.; Mogilnaya, O.A.; Stom, D.I.; Kudryasheva,
597 N.S. Bioluminescence as a tool for studying detoxification processes in metal salt solutions involving
598 humic substances. *J. Photochem. Photobiol. B* **2012**, *117*, 164–170, doi:10.1016/j.jphotobiol.2012.09.020.
599 82. Kudryasheva, N.S.; Tarasova, A.S. Pollutant toxicity and detoxification by humic substances: Mechanisms
600 and quantitative assessment via luminescent Biomonitoring. *Environ. Sci. Pollut. Res. Int*. **2015**, *22*, 155–167,
601 doi:10.1007/s11356-014-3459-6.
602 83. Tarasova, A.S.; Stom, D.I.; Kudryasheva, N.S. Antioxidant activity of humic substances via bioluminescent
603 monitoring in vitro. *Environ. Monit. Assess*. **2015**, *187*, 89, doi:10.1007/s10661-015-4304-1.
604 84. Kudryasheva, N.S.; Kovel, E.S.; Sachkova, A.S.; Vorobeva, A.A.; Isakova, V.G.; Churilov, G.N.
605 Bioluminescent enzymatic assay as a tool for studying antioxidant activity and toxicity of bioactive
606 compounds. *J. Photochem. Photobiol.* **2017**, *93*, 536–540, doi:10.1111/php.12639.
607 85. Sachkova, A.S.; Kovel, E.S.; Churilov, G.N.; Guseynov, O.A.; Bondar, A.A.; Dubinina, I.A.; Kudryasheva,
608 N.S. On mechanism of antioxidant effect of fullerenols. *Biochem. Biophys. Rep*. **2017**, *9*, 1–8,
609 doi:10.1016/j.bbrep.2016.10.011.
610 86. Sachkova, A.S.; Kovel, E.S.; Churilov, G.N.; Stom, D.I.; Kudryasheva, N.S. Biological activity of carbonic
611 nano-structures — Comparison via enzymatic bioassay. *J. Soils Sediments* **2018**,
612 doi:10.1007/s11368-018-2134-9.
613 87. Kovel, E.S.; Sachkova, A.S.; Vnukova, N.G.; Churilov, G.N.; Knyazeva, E.M.; Kudryasheva, N.S.
614 Antioxidant Activity and Toxicity of Fullerenols via Bioluminescence Signaling: Role of Oxygen
615 Substituents. *Int. J. Mol. Sci.* **2019**, *20*, 2324, doi:10.3390/ijms20092324.

616 88. Kleinhempel, D. Ein beitrag zur theories des huminstoffzustandes. *Arch. Agron. Soil Sci.* **1970,** *14*, 3-14,
617 doi:10.1080/03650347009412655.
618 89. Kudryasheva, N.; Vetrova, E.; Kuznetsov, A.; Kratasyuk, V.; Stom, D. Bioluminescent assays: Effects of
619 quinones and phenols. *Ecotoxicol. Environ. Saf.* **2002**, *53*, 221–225, doi:10.1006/eesa.2002.2214.
620 90. Brisebois, P.P.; Arnold, A.A.; Chabre, Y.M.; Roy, R.; Marcotte, I. Comparative study of the interaction of
621 fullerenol nanoparticles with eukaryotic and bacterial model membranes using solid-state NMR and FTIR
622 spectroscopy. *Eur. Biophys. J.* **2012**, *41*, 535-544, doi: 10.1007/s00249-012-0809-5.
623 91. Voeikov, V.L.; Yablonskaya, O.I. Stabilizing effects of hydrated fullerenes C_{60} in a wide range of
624 concentrations on luciferase, alkaline phosphatase, and peroxidase in vitro. *Electromagn. Biol. Med*. **2015**,
625 *34*, 160–166, doi:10.3109/15368378.2015.1036077.
626 92. Yablonskaya, O.I.; Ryndina, T.S.; Voeikov, V.L.; Khokhlov, A.N. A paradoxal effect of hydrated
627 C60-fullerene in an ultralow concentration on the viability and aging of cultivated Chinese hamster cells.
628 *Moscow Univ. Biol. Sci. Bull.* **2013**, *68*, 63-68, doi: 10.3103/S0096392513020107.
629 93. Bensasson, R.V.; Bretteich, M.; Frederiksen, J.; Gottinger, H.; Hirsch, A.; Land, E.J.; Leach, S.;
630 McGarvey, D.J.; Schonberger, H. Reactions of e-aq, CO_2^-, HO^-, O_2^- and $O_2(^1Dg)$ with a dendro[60]fullerene
631 and $C_{60}[C(COOH)_2]n$ (n = 52-6). *Free Radic. Biol. Med*. **2000**, *29*, 26-33, doi:10.1016/s0891-5849(00)00287-2.