**The Comparative Toxic Impact Assessment of Carbon Nanotubes, Fullerene, Graphene, and Graphene Oxide in Marine microalgae Porphyridium purpureum**

**Konstantin Pikula 1, Seyed Ali Johari 2, Ralph Santos-Oliveira 3,4 and Kirill Golokhvast 1,5**

1 Polytechnical Institute, Far Eastern Federal University, 10 Ajax Bay, Russky Island, 690922 Vladivostok, Russia; pikula\_ks@dvfu.ru (K.P.)

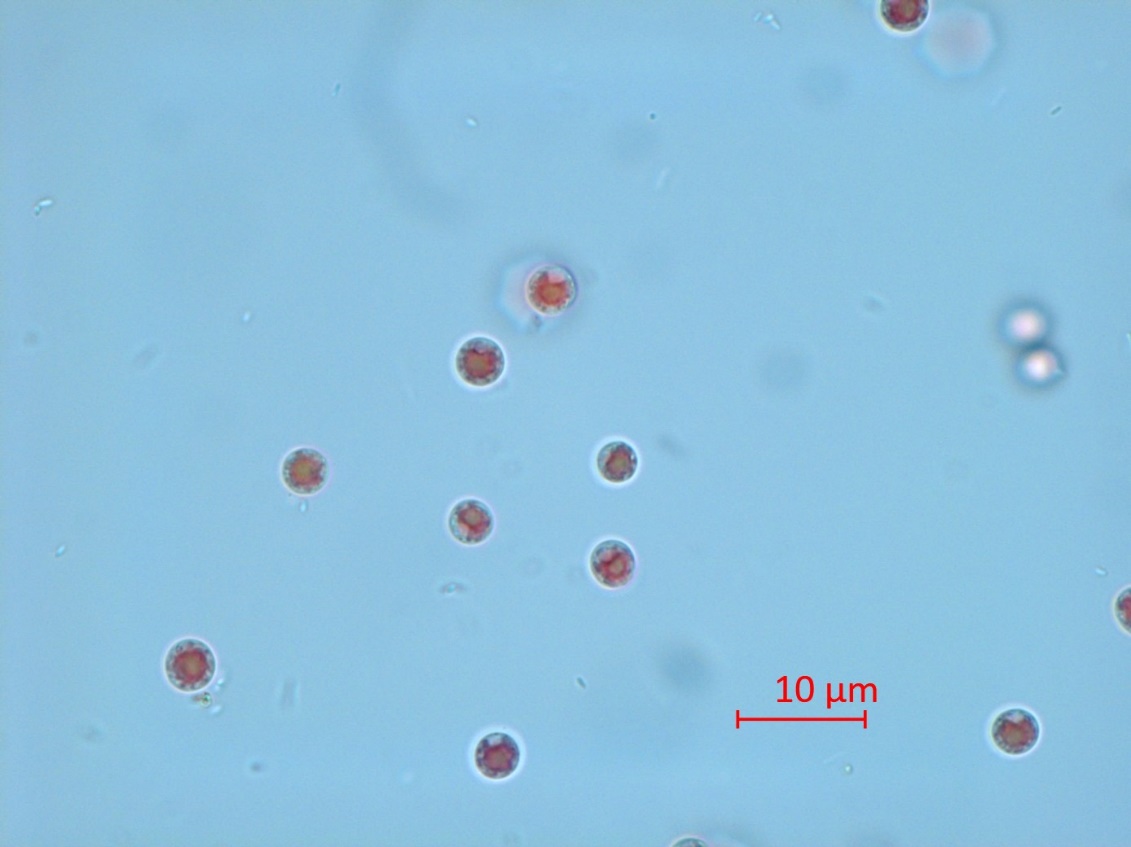
2 Department of Fisheries, Faculty of Natural Resources, University of Kurdistan, Pasdaran St, Sanandaj 66177-15175, Iran; sajohari@gmail.com (S.A.J.)

3 Laboratory of Nanoradiopharmaceuticals and Synthesis of Novel Radiopharmaceuticals, Nuclear Engineering Institute, Brazilian Nuclear Energy Commission, Rua Hélio de Almeida 75, 21941906 Rio de Janeiro, Brazil; roliveira@ien.gov.br (R.S.O.)

4 Laboratory of Nanoradiopharmaceuticals and Radiopharmacy, Rio de Janeiro State University, R. São Francisco Xavier, 524, 23070200 Rio de Janeiro, Brazil

5 Siberian Federal Scientific Centre of Agrobiotechnology, Centralnaya Str., Presidium, 633501 Krasnoobsk, Russia; golokhvast@sfsca.ru (K.G.)

**\*** Correspondence: pikula\_ks@dvfu.ru



**Figure S1.** Microscopic picture of *P. purpureum* from control group.

Table S1: The statistical significance calculation of esterase activity, membrane potential, and ROS generation in *P. purpureum* cells after 24 h of exposure; Table S2: The statistical significance calculation of the changes in *P. purpureum* cells after 96 h of exposure.

**Table S1.** The statistical significance calculation of esterase activity, membrane potential, and ROS generation in P. purpureum cells after 24 h of exposure.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Concentration. mg/L** | **CNTs** | | **C60** | | **Gr** | | **GrO** | |
| **Growth rate** | | | | | | | | |
| 1 | \*\*\* | 0.0004 | ns | 0.6562 | ns | 0.4119 | \*\*\*\* | <0.0001 |
| 10 | \*\*\*\* | <0.0001 | ns | 0.7143 | ns | 0.0883 | \*\*\*\* | <0.0001 |
| 25 | \*\*\*\* | <0.0001 | ns | 0.6845 | \*\* | 0.0049 | \*\*\*\* | <0.0001 |
| 50 | \*\*\*\* | <0.0001 | ns | 0.2042 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 |
| 75 | \*\*\*\* | <0.0001 | \* | 0.0454 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 |
| 100 | n/a | <0.0001 | \*\* | 0.0057 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 |
| 125 | n/a | <0.0001 | \* | 0.0118 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 |
| **Esterase activity** | | | | | | | | |
| 1 | \* | 0.0224 | \*\*\* | 0.0007 | ns | 0.2676 | ns | 0.9998 |
| 10 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 | \*\* | 0.0018 | \*\* | 0.0032 |
| 25 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 |
| 50 | \*\*\*\* | <0.0001 | ns | 0.2647 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 |
| 75 | \*\*\*\* | <0.0001 | ns | 0.1285 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 |
| 100 | n/a | <0.0001 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 |
| 125 | n/a | <0.0001 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 |
| **Membrane potential** | | | | | | | | |
| 1 | ns | 0.2576 | ns | 0.1437 | ns | 0.1169 | ns | 0.1001 |
| 10 | ns | 0.9020 | \* | 0.0148 | \*\*\* | 0.0004 | ns | 0.0617 |
| 25 | ns | 0.1008 | \* | 0.0207 | \*\*\*\* | <0.0001 | ns | 0.3921 |
| 50 | \*\*\*\* | <0.0001 | \*\* | 0.0020 | \*\*\*\* | <0.0001 | ns | 0.9951 |
| 75 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 | \*\*\* | 0.0009 | \* | 0.0106 |
| 100 | n/a | <0.0001 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 | \*\* | 0.0024 |
| 125 | n/a | <0.0001 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 | \*\* | 0.0067 |
| **ROS generation** | | | | | | | | |
| 1 | ns | 0.6425 | ns | 0.4558 | ns | 0.6279 | ns | 0.0756 |
| 10 | ns | 0.9997 | ns | 0.4631 | ns | 0.5122 | ns | 0.1290 |
| 25 | \*\*\* | 0.0002 | ns | 0.3701 | ns | 0.2023 | ns | 0.1007 |
| 50 | \*\*\* | 0.0004 | ns | 0.9998 | \*\*\*\* | <0.0001 | \*\* | 0.0029 |
| 75 | \*\*\* | 0.0003 | ns | 0.2486 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 |
| 100 | n/a | 0.0109 | ns | 0.6707 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 |
| 125 | n/a | 0.0337 | ns | 0.9997 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 |

ROS, Reactive oxygen species; \*, p < 0.05; \*\*, p < 0.005; \*\*\*, p < 0.0005; \*\*\*\*, p < 0.0001; n/a, not assessed; ns, nonsignificant (p > 0.05).

**Table S2.** The statistical significance calculation of esterase activity, membrane potential, and ROS generation in P. purpureum cells after 24 h of exposure.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Concentration. mg/L** | **4-6 µm** | | **6-10 µm** | | **10-15 µm** | | |
| **CNTs** | | | | | | |
| 1 | ns | 0,7792 | ns | 0,7875 | ns | 0,9993 | |
| 10 | ns | 0,7496 | ns | 0,8065 | ns | 0,3760 | |
| 25 | \*\*\* | 0,0002 | \*\*\* | 0,0001 | ns | 0,5322 | |
| 50 | \*\*\*\* | <0,0001 | \*\*\* | 0,0001 | ns | 0,9988 | |
| 75 | n/a | n/a | n/a | n/a | n/a | n/a | |
| 100 | n/a | n/a | n/a | n/a | n/a | n/a | |
| 125 | n/a | n/a | n/a | n/a | n/a | n/a | |
| **C60** | | | | | | |
| 1 | \* | 0.0404 | \* | 0.0390 | ns | 0.9978 | |
| 10 | \* | 0.0184 | \* | 0.0178 | ns | 0.8838 | |
| 25 | ns | 0.5608 | ns | 0.5615 | ns | 0.8495 | |
| 50 | ns | 0.9631 | ns | 0.9589 | ns | 0.9137 | |
| 75 | ns | 0.8700 | ns | 0.8708 | ns | 0.9589 | |
| 100 | ns | 0.9924 | ns | 0.9919 | ns | 0.9997 | |
| 125 | ns | 0.4260 | ns | 0.4262 | ns | 0.8115 | |
| **Gr** | | | | | | |
| 1 | \*\* | 0.0090 | \*\* | 0.0088 | ns | 0.9999 | |
| 10 | ns | 0.5525 | ns | 0.5537 | ns | 0.9936 | |
| 25 | ns | 0.3239 | ns | 0.3188 | ns | 0.9977 | |
| 50 | \*\* | 0.0018 | \*\* | 0.0018 | ns | 0.9977 | |
| 75 | \*\* | 0.0029 | \*\* | 0.0028 | ns | 0.9317 | |
| 100 | \*\* | 0.0013 | \*\* | 0.0013 | ns | 0.9999 | |
| 125 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 | ns | 0.7740 | |
| **GrO** | | | | | | |
| 1 | ns | 0.1397 | ns | 0.1320 | ns | 0.9999 | |
| 10 | ns | 0.9784 | ns | 0.9911 | ns | 0.5981 | |
| 25 | \*\* | 0.0019 | \*\* | 0.0028 | \* | 0.0183 | |
| 50 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 | \*\*\*\* | <0.0001 | |
| 75 | \*\*\*\* | <0.0001 | \*\*\* | 0.0001 | \*\*\*\* | <0.0001 | |
| 100 | \*\*\* | 0.0003 | \*\* | 0.0019 | \*\*\*\* | <0.0001 | |
| 125 | \*\*\* | 0.0003 | \*\* | 0.0089 | \*\*\*\* | <0.0001 | |

ROS, Reactive oxygen species; \*, p < 0.05; \*\*, p < 0.005; \*\*\*, p < 0.0005; \*\*\*\*, p < 0.0001; n/a, not assessed; ns, nonsignificant (p > 0.05).