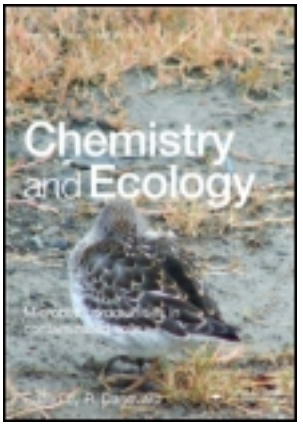

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Antioxidant responses in the polychaete *Perinereis gualpensis* (Nereididae) exposed to the carbon nanomaterial fullerene (C_{60})

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The objective of this study was to analyse biochemical responses induced by the carbon nanomaterial fullerene (C_{60}) in the polychaete *Perinereis gualpensis* (Nereididae). The activity of glutathione-*S*-transferase (GST), glutathione reductase (GR) and glutamate cysteine ligase (GCL), as well as total antioxidant capacity, concentration of glutathione (GSH) and malondialdehyde (TBARS), were analysed. Estuarine worms were maintained in sediments collected at an unpolluted site and spiked with fullerene ($3\ mg\ C_{60} \cdot g^{-1}$ sediment). A control group was run in parallel. Scanning electron microscope (SEM) images of sediment and fullerene indicated that the size of the carbon nanomaterial should enable it to be ingested by the polychaete. No evidence of oxidative damage (TBARS) was observed in any of the treatments, and the same was true for GSH and GCL measurements ($p > 0.05$). Total antioxidant capacity was higher in the C_{60} group after 2 and 7 d when compared with the control group ($p < 0.05$), suggesting that fullerene is acting as an antioxidant. The fact that *P. gualpensis* is an infaunal organism diminishes the chance of fullerene photoexcitation with consequent reactive oxygen species production. Thus, the data indicated an absence of toxic responses mediated by oxidative stress in estuarine worms exposed to C_{60} mixed in sediments.

Keywords: nanotoxicology; fullerene; antioxidant capacity; sediments; polychaete

The rapid development of nanotechnology holds the promise of benefits in several fields, including human health, with many products already present in the market. By contrast, significant concerns have been raised about the potential toxicity of these materials to human and environmental health [1,2]. In terms of impacts on the aquatic environment, the toxic responses to nanomaterials have been tested in a limited number of aquatic animals, mainly in fish and microcrustaceans such as *Daphnia magna* [3–5]. Existing information on benthic estuarine organisms is very scarce [6]. Some authors have pointed out that benthic species may be exposed to nanomaterials that aggregate and sink to the bottom [4]. In this scenario, polychaetes may be considered model animals for

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testing nanomaterial toxicity. Several field and laboratory studies with Nereidid polychaetes such as *Laeonereis acuta* and *Perinereis gualpensis* showed conspicuous antioxidant and oxidative damage responses, indicating the feasibility of analysing aquatic pollution through these benthic organisms [7–9].

Conflicting results have been reported for the carbon nanomaterial fullerene: some studies have shown antioxidant effects [10], whereas others reported induction of antioxidant responses or oxidative damage in aquatic organisms [11]. However, several studies have raised the possibility that fullerene toxicity is in fact due to organic solvents such as tetrahydrofuran (THF) which are employed in preparing fullerene suspensions [12]. Another factor that greatly influences fullerene toxicity is UV/VIS radiation which photoexcites this nanomaterial, leading to the generation of reactive oxygen species (ROS) that induce oxidative damage [13,14]. Taking into account the factors that alter the behaviour and effects of fullerene and also the fact that high ionic strength favours fullerene aggregation and precipitation [15], the objective of this study is to analyse biochemical responses in the polychaete *P. gualpensis* after chronic exposure to C_{60} mixed in sediment.

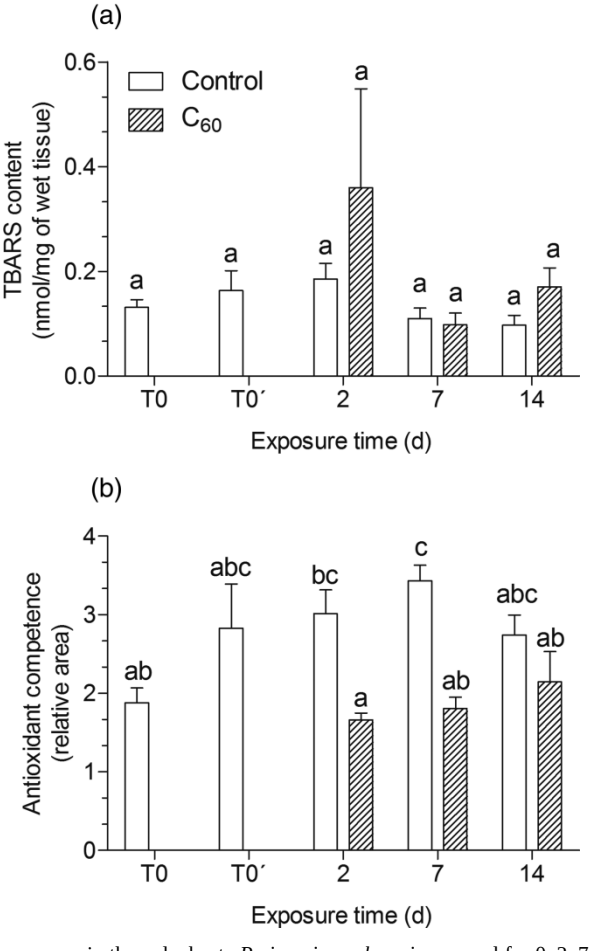

Figure 1. Biochemical responses in the polychaete *Perinereis gualpensis* exposed for 0, 2, 7 and 14 d to 0 or 3 mg of fullerene (C_{60}) per gram of sediment. In all cases, data are expressed as mean +1 SD. There were four or five analysed samples for each treatment. Identical letters indicate the absence of statistical differences ($p > 0.05$). (a) Concentration of thiobarbituric acid reactive substances (TBARS) (nmol·mg^{-1} of wet tissue). (b) Total antioxidant capacity against peroxyl radicals (relative area units).

The methods used followed the protocols employed in previous studies of aquatic organisms, including total antioxidant capacity against peroxyl radicals by fluorometry [16]. The relative difference between ROS concentration with and without peroxyl radicals was considered a measure of antioxidant capacity, with a high area difference (high ROS fluorescence production) indicating low antioxidant capacity, because high fluorescence levels result from the generation of peroxyl radicals (in other words, a low ability to neutralise peroxyl radicals).

Determination of the concentration of thiobarbituric reactive substances (TBARS) and reduced glutathione and glutathione reductase (GR), glutathione-*S*-transferase (GST) and glutamate cysteine ligase (GCL) activity was performed according to a previous study performed on the same polychaete species [9]. Worms (average weight: 0.31 ± 0.07 g) were collected at Raqui estuary, Chile (37° 13′ S; 73° 26′ W), which is characterised by a low anthropogenic impact in terms of low levels of polycyclic aromatic hydrocarbons (PAHs; $103\,ng{\cdot}g^{-1}$ dry weight) and heavy metals such as Hg ($0.085\,mg{\cdot}kg^{-1}$ dry weight) with respect to other estuaries in the area (Diaz-Jaramillo, unpublished data). Estuarine worms were kept in sediments from the same unpolluted site and with filtered seawater (salinity: 20) for 48 h. Subsequently, each worm was transferred to

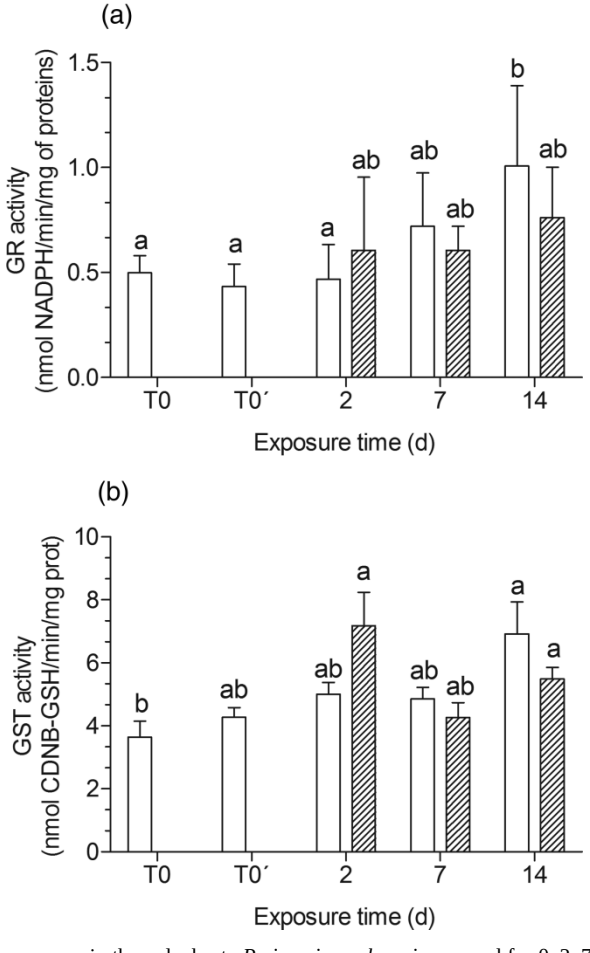

Figure 2. Biochemical responses in the polychaete *Perinereis gualpensis* exposed for 0, 2, 7 and 14 d to 0 or 3 mg of fullerene (C_{60}) per gram of sediment. In all cases data are expressed as mean +1 SD. There were four or five analysed samples for each treatment. Identical letters indicate the absence of statistical differences ($p > 0.05$). (a) Specific activity of glutathione reductase (nmols $NADPH{\cdot}min^{-1}{\cdot}mg^{-1}$ protein). (b) Specific activity of glutathione-*S*-transferase (GST) (nmol conjugated $CDNB{\cdot}min^{-1}{\cdot}mg^{-1}$ protein). GSH, reduced glutathione; CDNB, 1-chloro-2.4-dinitrobenzene.

an individual 200 mL plastic recipient with filtered seawater for 48 h to allow the worms to clean their gut contents. For the exposure experiment, the sediment collected in the unpolluted site was dried at 80 °C for 48 h in order to mix it uniformly with the nano compound and to eliminate other organisms. The sediment was also sieved through 400 μm mesh to exclude gross sediment particles. The final sediment presented $1.88 \pm 0.29\%$ organic matter and 1.90 ± 0.12 phi mean grain size. Next, the sediment was spiked with dried fullerene C_{60} particles (average C−C distance, 1.44 Å; mean ball diameter of C_{60}, 6.83 Å; mass density, 1.72 $g \cdot cm^{-3}$; www.sesres.com) and mixed in a horizontal shaker during 48 h to obtain a final concentration of 3 mg $C_{60} \cdot g^{-1}$ of sediment.

For the exposure experiment, worms were placed in the plastic recipient containing 200 g of C_{60}-spiked sediment plus 10 cm of filtered seawater. A control group was run in parallel with the same sediment without C_{60}. Biochemical responses were evaluated in animals frozen in liquid N_2 after field collection (T0); after transport to the laboratory packed in ice (T0′); and after 2, 7 and 14 d of C_{60} exposure, following Petersen et al. [17], who obtained maximum fullerene bioaccumulation rates in annelids after two weeks of exposure. The physicochemical parameters of the filtered

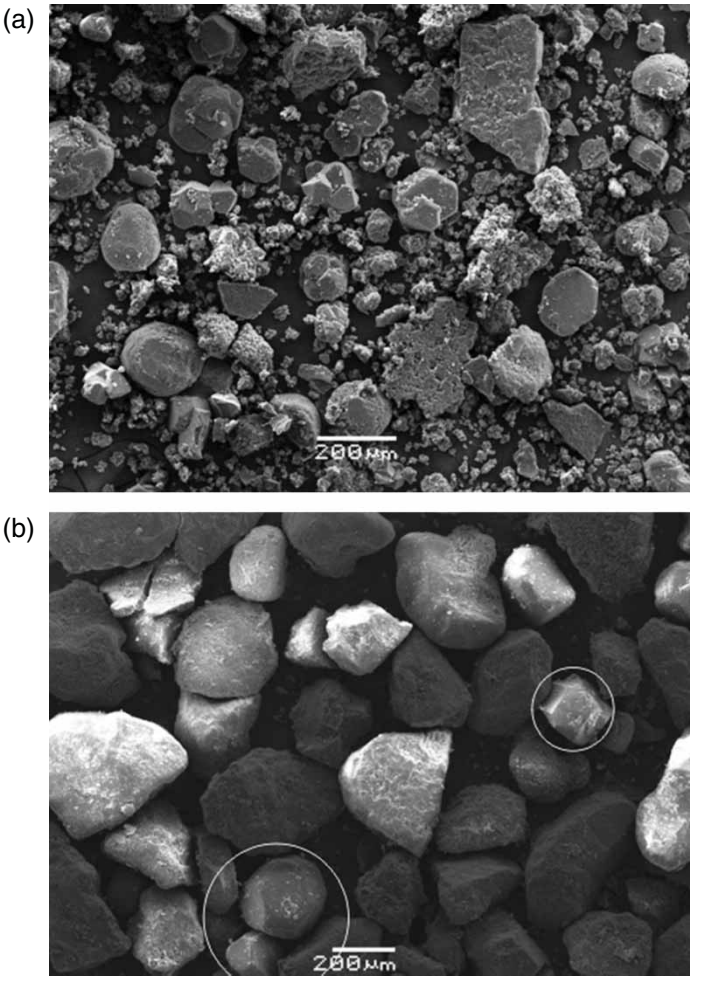

Figure 3. Scanning electron microscope (SEM) images of: (a) aggregates of fullerene (C_{60}) without sediments; (b) sediments with putative aggregates of fullerene (C_{60}) (in circles). Scale: 200 μm.

seawater were recorded during the experiment (C_{60}: pH 7.77 ± 0.02, $T°$ 14.97 ± 0.15 °C; Control: pH 7.71 ± 0.02, $T°$ 15.40 ± 0.20 °C). Scanning electronic microscopy was performed in fullerene and sediment spiked with fullerene. Samples were coated with a 500 Å-thick gold film and analysed with a SEM JEOL LV 6380 microscope. Statistical data analysis was performed using a one-way analysis of variance (ANOVA) [18], where the treatments compared were T0, T0′, control (2 d), C_{60} (2 d), control (7 d), C_{60} (7 d), control (14 d) and C_{60} (14 d). A significance level of 5% was adopted.

No evidence of oxidative damage (TBARS) was found for any of the treatments ($p > 0.05$; Figure 1a), and the same was true for GSH and GCL measurements ($p > 0.05$; data not shown). Total antioxidant capacity against peroxyl radicals was higher in the C_{60} group after 2 and 7 d when compared with the control group ($p < 0.05$; Figure 1b). After 14 d, higher GR activity was observed in the control group with respect to T0 ($p < 0.05$) which did not occur in the C_{60} group (Figure 2a). The GST activity increased in both treatments after 14 d ($p < 0.05$; Figure 2b) when compared with the T0 group, although the response was quicker in the fullerene-exposed group, where high GST activity was detected at day 2 (Figure 2b).

The results suggest the absence of toxic responses in estuarine worms exposed to C_{60}. The same was observed in the polychaete *A. marina* exposed to a similar concentration of carbon nanotubes [6]. It is possible that worms hidden in the sediment are less prone to photo-oxidation processes than epibentic organisms when exposed to the superoxidant promoter condition implied by fullerene under UV/VIS radiation [13]. Also, it may be inferred that a pro-oxidant response is triggered under laboratory conditions, as suggested by higher GR activity and lower total antioxidant capacity in the control group after 2 and 7 d. These effects were not observed in the C_{60} group, indicating that fullerene acts as an antioxidant, as noted by Gharbi et al. [10].

In summary, according to the protocol employed in this study, it may be concluded that *P. gualpensis* may incorporates fullerene particles of similar size to the sediment used, as shown in Figure 3. The potential toxicity of fullerene for benthic organisms seems to be negligible, possibly due to the lack of photoexcitation.

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References

[1] R. Owen and M. Depledge, *Nanotechnology and the environment: risk and rewards*, Mar. Pollut. Bull. 50 (2005), pp. 609–612.
[2] A.D. Maynard, R.J. Aitken, T. Butz, V. Colvin, K. Donaldson, G. Oberdörster, M.A. Philbert, J. Ryan, A. Seaton, V. Stone, S.S. Tinkle, L. Tran, N.J. Walker, and D.B. Warheit, *Safe handling of nanotechnology*, Nature 444 (2006), pp. 267–269.
[3] S.B. Lovern, J.R. Strickler, and R. Klaper, *Behavioral and physiological changes in* Daphnia magna *when exposed to nanoparticle suspensions (titanium dioxide, nano-C_{60} and $C_{60}HxC_{70}Hx$)*, Environ. Sci. Technol. 41 (2007), pp. 4465–4470.
[4] A. Baun, N.B. Hartmann, K. Grieger, and K.O. Kusk, *Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity test*, Ecotoxicology 17 (2008), pp. 387–395.
[5] C.Y. Usenko, S.L. Harper, and R.L. Tanguay, *Fullerene C_{60} exposure elicits an oxidative stress response in embryonic zebrafish*, Toxicol. Appl. Pharmacol. 229 (2008), pp. 44–55.
[6] T. Galloway, C. Lewis, I. Dolciotti, B.D. Johnston, J. Moger, and F. Regoli, *Sublethal toxicity of nano-titanium dioxide and carbon nanotubes in a sediment dwelling marine polychaete*, Environ. Pollut. 158 (2009), pp. 1748–1755.
[7] M. Ferreira-Cravo, F. Reinhardt Piedras, T. Barros Moraes, J.L. Ribas Ferreira, D.P. Salomão de Freitas, M. Dornelles Machado, L.A. Geracitano, and J.M. Monserrat, *Antioxidant responses and reactive oxygen species generation*

in different body regions of the estuarine polychaeta Laeonereis acuta *(Nereididae)*, Chemosphere 66 (2007), pp. 1367–1374.

[8] M. Ferreira-Cravo, J. Ventura-Lima, J. Zomer Sandrini, L.L. Amado, L.A. Geracitano, M. Rebelo, A. Bianchini, and J.M. Monserrat, *Antioxidant responses in different body regions of the polychate* Laeonereis acuta *(Nereididae) exposed to copper*, Ecotoxicol. Environ. Saf. 72 (2009), pp. 388–393.

[9] M. Diaz-Jaramillo, J.L. Ferreira, L.L. Amado, J. Ventura-Lima, A. Martins, M.R. Retamal, R. Urrutia, C. Bertrán, R. Barra, and J.M. Monserrat, *Biomonitoring of antioxidant and oxidative stress responses in* Perinereis gualpensis *(Polychaeta: Nereididae) in Chilean estuarine regions under different anthropogenic pressure*, Ecotoxicol. Environ. Saf. 73 (2010), pp. 515–523.

[10] N. Gharbi, M. Pressac, M. Hadchouel, H. Szwarc, S.R. Wilson, and F. Moussa, *Fullerene is a powerful antioxidan* in vivo *with no acute or subacute toxicity*, Nano Lett. 5 (2005), pp. 2578–2585.

[11] R. Klaper, J. Crago, J. Barr, D. Arndt, K. Setyowati, and J. Chen, *Toxicity biomarker expression in daphnids exposed to manufacturated nanoparticles: changes in toxicity with functionalization*, Environ. Pollut. 157 (2009), pp. 1152–1156.

[12] P. Spohn, C. Hirsch, F. Hasler, A. Bruinink, H.F. Krug, and P. Wick, *C_{60} fullerene: a powerful antioxidant or a damaging agent? The importance of an in-depth material characterization prior to toxicity assays*, Environ. Pollut. 157 (2009), pp. 1134–1139.

[13] J.P. Kamat, T.P.A. Devasagayam, K.I. Proyadarsini, and H. Mohan, *Reactive oxygen species mediated membrane damage induced by fullerene derivatives and its possible biological implications*, Toxicology 155 (2000), pp. 55–61.

[14] N. Shinohara, T. Matsumoto, M. Gamo, A. Miyauchi, S. Endo, Y. Yonezawa, and J. Nakanishi, *Is lipid peroxidation induced by the aqueous suspension of fullerene C_{60} nanoparticles in the brains of* Cyprinus carpio*?* Environ. Sci. Technol. 43 (2009), pp. 948–953.

[15] S. Deguchi, T. Yamazaki, S. Mukai, R. Usami, and K. Horikoshi, *Stabilization of C_{60} nanoparticles by protein adsorption and its implications for toxicity studies*, Chem. Res. Toxicol. 20 (2007), pp. 854–858.

[16] L.L. Amado, M.L. Garcia, P.B. Ramos, R.F. Freitas, B. Zafalon, J.L.R. Ferreira, J.S. Yunes, and J.M. Monserrat, *A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: application to evaluate microcystins toxicity*, Sci. Total Environ. 407 (2009), pp. 2115–2123.

[17] E. Petersen, Q. Huang, and W. Weber, *Ecological uptake and depuration of carbon nanotubes by* Lumbriculus variegates, Environ. Health Perspect. 116 (2008), pp. 496–500.

[18] J. Zar, *Biostatistical Analysis*, Prentice Hall, Englewood Cliffs, NJ, 1984.