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Screening evaluation of the ecotoxicity and genotoxicity of soils contaminated with organic and inorganic nanoparticles: The role of ageing

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ABSTRACT

This study aimed to evaluate the toxicity and genotoxicity of soils, and corresponding elutriates, contaminated with aqueous suspensions of two organic (vesicles of sodium dodecyl sulphate/didodecyl dimethylammonium bromide and of monoolein and sodium oleate) and five inorganic nanoparticles (NPs) (TiO_2, $TiSiO_4$, CdSe/ZnS quantum dots, Fe/Co magnetic fluid and gold nanorods) to *Vibrio fischeri* and *Salmonella typhimurium* (TA98 and TA100 strains). Soil samples were tested 2 h and 30 days after contamination. Suspensions of NPs were characterized by Dynamic Light Scattering. Soils were highly toxic to *V. fischeri*, especially after 2 h. After 30 days toxicity was maintained only for soils spiked with suspensions of more stable NPs (zeta potential > 30 mV or <−30 mV). Elutriates were particularly toxic after 2 h, except for soil spiked with Fe/Co magnetic fluid, suggesting that ageing may have contributed for degrading the organic shell of these NPs, increasing the mobility of core elements and the toxicity of elutriates. TA98 was the most sensitive strain to the mutagenic potential of soil elutriates. Only elutriates from soils spiked with gold nanorods, quantum dots (QDs) and $TiSiO_4$ induced mutations in both strains of *S. typhimurium*, suggesting more diversified mechanisms of genotoxicity.

1. Introduction

The application of regulatory schemes for the authorization of NPs requires different levels of information, namely those concerning environmental hazards of NPs to biota from the different environmental compartments and exposures pathways [1]. Till now these aspects have been difficultly assessed, at least for the terrestrial compartment. This is mainly attributed to the lack of separation and analytical methods able to quantify environmental concentrations and to characterize the physical status, the adsorption/desorption, the precipitation and the dissolution of NPs after being released into the soil [2,3]. Future emission rates are still unknown and few ecotoxicological data exists for the terrestrial compartment [2] compromising our ability to predict their impact on terrestrial communities. Existing information is limited both to a few number of species and NPs [4–21]. Even fewer have focused the mobility and transport of NPs into the soil matrix [7,22,23]. Despite the availability of more than 1000 nanocompounds in the market [24], with different sizes, shapes, surface area, surface functions and chemical composition, the toxicity of most of them remains unknown, and probably each one will require a different ecological risk assessment (ERA) [25].

The Microtox® is an acute assay that measures the effect of toxicants and environmental samples on light production by the bacteria *Vibrio fischeri*. Despite being initially developed for liquid samples [26], now the Microtox assay can be used for testing solid samples (soil and sediments) and has wide application in the screening step of ERA schemes. The Ames assay is also a short-term assay, usually applied, with and without metabolic activation by the liver homogenate (S9) for the screening evaluation of the mutagenic potential of soil samples in *Salmonella typhimurium* [e.g. 27]. Since the Microtox® and Ames assays require an extraction step they may give an idea about the mobility of potential toxic/mutagenic compounds, from soils to the groundwater.

Hence, this paper is focused in a screening evaluation of the eco- and genotoxicity of soils spiked with two organic and five inorganic NPs (having a vast array of commercial and biomedical applications – cosmetics, coatings, medical imaging) and corresponding soil elutriates, using two tests with bacteria (Microtox® and Ames test). In addition the effect of soil ageing in the toxicity of NPs was analysed.

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2. Material and methods

2.1. Nanomaterials tested

Two organic NPs were tested in this study: (i) vesicles of sodium dodecyl sulphate and didodecyl dimethylammonium bromide – SDS/DDBA (particle size 30 nm) [28] purchased from Sigma–Aldrich and (ii) vesicles of monoolein and sodium oleate – Mo/NaO (particle size 60 nm) [29] purchased from Danisco Ingredients (Braband, Denmark) and Nu-Chek Prep, Inc. (Elysian, MN, USA). For the inorganic NPs, five compounds were tested: (i) titanium dioxide – TiO_2 (particle size < 100 nm, 99.9% metal basis); (ii) titanium silicon oxide – $TiSiO_4$ (particle size < 50 nm, 99.8% of purity); (iii) quantum dots (QDs) Lumidot™ CdSe/ZnS 530 (5 mg mL^{-1} in toluene); (iv) Fe/Co magnetic fluid stabilized with cashew shell liquid (CNSL) (0.19% v/v) in toluene (average particle size 7 nm) and (v) gold nanorods (axial diameter 10 nm, length 35 nm, longitudinal Surface Plasmon Resonance peak 750 nm, wt. concentration 33.4 μg L^{-1}) in deionised (DI) water with <0.1% ascorbic acid and 0.1% of cetyltrimethylammonium bromide surfactant capping agent. The inorganic NPs were supplied by Sigma–Aldrich (for the first three), STREM Chemicals Inc. (Bischheim, France) and Nanopartz™ (Salt Lake City, UT, USA), respectively.

2.2. Characterization of NPs aqueous suspensions

Each NP aqueous suspension was characterized for size, size distribution, and particle surface potential (zeta potential, ζ) through Dynamic Light Scattering (DLS) using a Zeta Sizer Nano ZS, Zen 3500, with a 532 nm laser (Malvern Instruments, UK).

All the measurements were made at 20 °C, the same temperature at which soil samples were incubated (please see experimental design section). Size and surface charge (ζ potential) parameters were calculated by the Zetasizer NanoSoftware, version 6.01, and reported in this work. Since large particles (e.g. dusts) and particle aggregates scatter light more intensively at a forward angle (12.8°), all the measurements were made using a backscatter angle (173°). Aggregation index relates the average diameter measured with the backscatter angle with the one measured with the forward scatter angle. If no aggregation exists both values are equal and the aggregation index is zero. The polydispersity index (PdI) is a measure of distribution of particle sizes. The magnitude of the zeta potential gives an indication of the stability of NPs in the suspension, being greater for values below or above −30 mV/+30 mV respectively [30].

2.3. Test soil

The standard artificial OECD soil [31] was used as test medium in this study. This soil was prepared by mixing sand, kaolin clay and sphagnum peat (4 mm sieved) in a 7:2:1 mass proportion, and by adjusting pH to 6.0 ± 0.5, with calcium carbonate. Afterwards moisture and maximum water holding capacity (WHC) of soil (both in percentage) were determined. Soil moisture was determined from the loss of weight of 5 soil replicates, after drying at 105 °C, for 24 h. WHC was determined according to the ISO guideline No. 17512-1 [32].

2.4. Experimental design

For each NP, three replicates of OECD soil (6 g per replicate) were weight and the soil water content was adjusted to 80% of its maximum WHC. The volume of water added was used to prepare suspensions of NPs and to disperse them on soil. Concentrations tested for each NP are described in Table 1. Information provided by the supplier of Fe/Co magnetic fluid (97% of toluene, 0.3% of CNSL and 2% of metallic compound) was not sufficient to determine the added dose (wt.) of both metallic elements, in terms of mass of the element per mass of soil. Hence the concentration of terms of volume of the fluid per dry mass of soil is given. Since no information is available about the ecological relevant concentrations of these NPs in the soil matrix, the concentrations tested in this work were based on those tested by Lopes et al. (unpublished data) as aqueous suspensions, using the same test species from this study. After spiking, each replicate was thoroughly mixed, to homogenize the distribution of the NPs suspension. Thereafter replicates were incubated at 20 ± 2 °C and at photoperiod of 16 h^L:8 h^D. Two hours later 1 g of soil was withdraw from each replicate and thoroughly mixed to give rise to a 3 g composite sample, which was used to prepare elutriates. Another 1 g of soil was removed from each replicate to obtain another composite soil sample, for each soil treatment. After 30 days, the same sampling procedure was followed and the eco- and genotoxicity of soils and elutriates was assessed by the same battery of assays. During the incubation period, soil moisture was checked and adjusted each 2 days.

Negative controls were prepared for both incubation periods (2 h and 30 days) incubating soils moistened either with water or with a suspension of toluene in water. Since toluene was in a concentration >90% in both Lumidot™ (CdSe/ZnS) and Fe/Co magnetic fluid composition, the concentration of toluene tested was 100 mL kg^{-1} (dw), similarly to the concentration of Lumidot™ tested.

2.5. Soil elutriates

To prepare elutriates, each composite soil sample from the different NPs treatments, was placed in a 50 mL Erlenmeyer flask, with of Milli-Q® water, in a proportion of 1:4 (m/v) and covered with aluminium foil. The flasks were shaken at 120 r.p.m., during 24 h, at 20 ± 2 °C. Afterwards, flasks were left to rest at room temperature, allowing large particles to settle. To sterilize the samples the supernatants were filtered through 0.2 μm cellulose acetate filters in a laminar flow clean bench.

2.6. Eco- and genotoxicity assays

The ecotoxicity of soils spiked with aqueous suspensions of NPs, and of corresponding soil elutriates, to *V. fischeri*, was assessed for 5, 15 and 30 min of exposure carrying out the 81.9% Basic test protocol [33]. For soil samples, the Basic Solid Phase test protocol was followed [33]. All tests were performed using the Microtox 500 Analyzer. The EC_{20} and EC_{50} (toxic effects thresholds) were computed for each soil and elutriate using the Software for MicrotoxOmni Azur [33]. To account for possible interferences in the luminescence measurements, absorbance was measured for each NP suspension, without the addition of the bacteria. For all NP suspensions absorbance was zero.

To assess NPs mutagenicity, soil elutriates were diluted with distilled water to obtain a range of dilutions to be tested (3.13, 6.25, 12.5, 25.0, 50.0 and 100%) corresponding to 5–0.156 mg soil equivalent per plate. The different soil elutriate concentrations were tested with both *S. typhimurium–his*$^-$ TA98 and TA100 strains, following the procedure described by Maron and Ames [34] and by Mortelmans and Zeiger [35] for the pre-incubation assay. Moreover, assays with both bacterial strains were performed with and without in vitro metabolic activation of pollutants with microsomal enzymes prepared from Sprague–Dawley (S9) liver induced with Aroclor 1254, purchased from Trinova Biochem™. Nitrofuratoin (TA100) and 2-nitrofluorene (TA98) were used as positive controls (10 μg/plate) in assays without S9, while 2-aminoanthracene (10 μg/plate) was used in assays with S9 for both strains. Distilled water was used in negative controls. Revertent colonies were

Table 1
DLS data for aqueous suspensions of NPs, prepared to spike the test soil.

	Concentration in the soil	Average size (nm)	PdI	Aggregation index	Zeta potential (mV)
SDS/DDBA	1.7 g kg^{-1}	71.75	0.273	1.58	−96.6
MO/NaO	5 g kg^{-1}	296.2	0.550	1.30	−72.8
TiO_2	5 g kg^{-1}	1425	0.896	−0.608	27.6
$TiSiO_4$	5 g kg^{-1}	738.9	1.0	−0.997	−5.92
Lumidot™ (CdSe/ZnS)	0.5 mg kg^{-1} (Cd or Zn)	2621	0.870	−0.119	−21.3
FeCo magnetic fluid	0.5 ml kg^{-1} [a]	585	0.397	1.61	−21.2
Gold nanorods	3.34 mg kg^{-1}	12.32	0.433	0.266	24.8

PdI stands for polydispersity index.
[a] Concentration expressed in terms of volume of the magnetic fluid per dry mass of soil.

counted after incubating plates for 48 h, at 37 °C. Results were considered positive, either if a dose-related increase in the number of revertent colonies was observed or when the average number of revertent colonies was higher than twice the number recorded in negative controls [35]. According to OECD guidelines [36], this last criterion is sufficient to assume mutagenic potential, even without a dose–effect relationship.

When suspicions about the toxicity of spiked soil elutriates to *S. typhimurium his*$^-$ strains occurred, one-way analysis of variance followed by a Dunnett test, were performed. These tests aimed to check for a significant reduction of revertent colonies, in comparison with negative control plates.

3. Results

The results from DLS analysis of aqueous suspensions of NPs, prepared to spike OECD soil samples, are described in Table 1. All the NPs showed the tendency to form large aggregates, except for SDS/DDBA and gold nanorods. In opposition TiO_2 and QDs, displayed a greater tendency to form aggregates larger than 1 μm, when dispersed in distilled water. Also both suspensions showed extremely high PdI values, suggesting a large range of aggregate sizes in suspension. Further the negative values for aggregation indexes, give an additional indication of NPs aggregation in the aqueous suspension. With respect to zeta potential only SDS/DDBA and Mo/NaO showed a value greater than −30/+30 mV, indicating high stability in suspension. The $TiSiO_4$ suspension showed a zeta potential value near zero, indicating that this was the less stable suspension hence the formation of even large aggregates should be expected.

3.1. *Microtox® assays of the whole soil matrix and elutriates*

Table 2 displays the EC_{20} and EC_{50} values determined after 5, 15 and 30 min of exposure, the corresponding 95% confidence intervals and, the EC_{50}/EC_{20} ratios, for the OECD soil samples spiked with organic and inorganic NPs, after the Solid Phase Microtox® assay. All soils collected 2 h after spiking inhibited the bioluminescence of *V. fischeri*. The classification of samples as toxic was based on that proposed by Kwan and Dutka [37]: samples with values of EC_{50} = <5000 mg L^{-1} are very toxic; samples with 5000 mg L^{-1} < EC_{50} = <10,000 mg L^{-1} are moderately toxic, and with EC_{50} > 10,000 mg L^{-1} are non toxic. The OECD soil was very to moderately toxic to *V. fischeri*, but the addition of toluene did not increased its toxicity because EC_{50} values were similar to those obtained for soil spiked with distilled water.

Soils spiked with suspensions of organic (SDS/DDBA and Mo/NaO) and inorganic NPs (gold nanorods and Fe/Co magnetic fluid) were very toxic to *V. fischeri*. The EC_{50} values recorded were lower than those recorded for the OECD with distilled water or toluene, suggesting that NPs may have increased the toxicity of soil. The EC_{50} values recorded for soils spiked with SDS/DDBA were similar for both incubation periods (2 h and 30 days). However, for the soils spiked with suspensions of Mo/NaO, gold nanorods, or Fe/Co magnetic fluid, the EC_{50} values increased, after 30 days of incubation, indicating a decrease in toxicity. Comparing the EC_{50}/EC_{20} ratios, the lowest values were recorded for the soils spiked with gold nanorods and with Fe/Co magnetic fluid, suggesting that, for the same exposure period, these contaminated soils took less time to exert their effects (Table 2). In general, the toxicity of soils spiked with SDS/DDBA or Mo/NaO was maintained or decreased, respectively, throughout the bacteria exposure period (from 5 to 30 min), while the toxicity of soils spiked with gold nanorods or with Fe/Co magnetic fluid increased with exposure period.

The soil spiked with a suspension of QDs (2 h) was also very toxic to *V. fischeri* since EC_{50} = <5000 mg L^{-1} were recorded for all the exposure periods (5–30 min). However, as the EC_{50} values were similar to those obtained for the OECD soil spiked with a suspension of toluene or with distilled water, such toxic effect may have been caused by soil components. Soils spiked with suspensions of titanium NPs were not toxic to *V. fischeri* (both at 2 h and 30 days).

For soil elutriates (Table 3), it was observed that those obtained from OECD soil spiked with suspensions of the two organic NPs and of Fe/Co magnetic fluid were toxic to *V. fischeri*. The other soil elutriates had some inhibitory effect in the bioluminescence of the bacteria, but always lower than 50%. For the elutriates of soils spiked with suspensions of both organic NPs the toxicity was extremely high, when these elutriates were obtained after 2 h of incubation, but it decreased or even disappeared after 30 days. Comparing the EC_{50}/EC_{20} ratios, the elutriate obtained from the OECD soil spiked with the Mo/NaO showed the lowest values suggesting that it exerts toxicity faster than the other elutriates. After 30 days of incubation this ratio showed a remarkable increase for all the exposure periods. Elutriates spiked with distilled water were not toxic to *V. fischeri* (Table 3) suggesting that the toxicity of elutriates mentioned above may have resulted from the toxicity of the aqueous suspensions of the vesicles (Lopes et al. unpublished data), used to spike the soils. Further, and in opposition to corresponding soils, the toxicity of elutriates increased from 5 to 30 min of exposure.

The elutriate of the OECD soil spiked with a suspension of toluene (30 days) inhibited the bioluminescence of *V. fischeri*, but always at a percentage lower than 50% (Table 3). This observation suggests that, to some extent, toluene may have been responsible by the toxicity of elutriates of soils spiked with QDs and Fe/Co magnetic fluid suspensions. However, the extremely low EC_{50} values recorded for the elutriate of OECD soil spiked with Fe/Co magnetic fluid (30 days) (Table 3) indicates that Fe/Co NPs may have also contributed to toxicity.

Table 2
Effective concentrations (mg/L) (95% CI, inside brackets) of the OECD soil spiked with the suspensions of NPs, and incubated for 2 h and 30 days, causing 20 and 50% inhibition in the bioluminescence (EC_{20} and EC_{50}, respectively) of *V. fischeri*.

Chemical	Test medium	Endpoint (mg L^{-1})	5 min	15 min	30 min
OECD soil + water	Soil (t = 2 h)	EC_{20}	412.5 (113.2–1505)	62.4 (2.26–1736)	NC (h.e. 87%)
		EC_{50}	3326 (2543–4350)	4844 (3579–6557)	5559 (4355–7096)
		EC_{50}/EC_{20}	8.06	77.6	–
	Soil (t = 30 days)	EC_{20}	NT	2426 (288.1–20,430)	NC (h.e. 77%)
		EC_{50}	NT	7126 (1695–29,970)	8663 (900.7–83,310)
		EC_{50}/EC_{20}	–	2.9	–
OECD soil + toluene	Soil (t = 2 h)	EC_{20}	6697 (1922–23,330)	6553 (2583–16,620)	4071 (2034–8147)
		EC_{50}	31,310 (5240–183,600)	25,700 (6996–94,400)	20,600 (8928–47,510)
		EC_{50}/EC_{20}	4.7	3.9	5.1
	Soil (t = 30 days)	EC_{20}	2892 (376.9–22,200)	5988 (62.69–57,180)	5508 (492.1–61,660)
		EC_{50}	5942 (1088–32,450)	6178 (1246–30,640)	6027 (1159–31,350)
		EC_{50}/EC_{20}	2.05	1.03	1.09
SDS/DDBA	Soil (t = 2 h)	EC_{20}	NC	NC	NC
		EC_{50}	1019 (474–2188)	1091 (482–2472)	1507 (706–3215)
		EC_{50}/EC_{20}	–	–	–
	Soil (t = 30 days)	EC_{20}	1120 (173.5–7228)	295.6 (16.33–5350)	201.1 (93.92–4308)
		EC_{50}	12,720 (3797–42,630)	3332 (878.6–12,630)	1741 (349–8681)
		EC_{50}/EC_{20}	11.4	11.3	8.6
Mo/NAO	Soil (t = 2 h)	EC_{20}	NC	NC	NC
		EC_{50}	723 (472–1160)	1039 (912–1184)	1035 (1005–1694)
		EC_{50}/EC_{20}	–	–	–
	Soil (t = 30 days)	EC_{20}	5174 (1422–18,820)	272.1 (9.7–7658)	212.4 (10.66–4235)
		EC_{50}	40,990 (3154–53,270)	4429 (1285–15,260)	2683 (740.2–9722)
		EC_{50}/EC_{20}	7.9	16.3	12.6
Lumidot (CdSe/ZnS)	Soil (t = 2 h)	EC_{20}	NC	NC	NC
		EC_{50}	3753 (3173–4440)	5016 (4239–5934)	5631 (4393–7219)
		EC_{50}/EC_{20}	–	–	–
	Soil (t = 30 days)	EC_{20}	10,780 (745.8–15,350)	NC	NC
		EC_{50}	13,120 (1481–11,620)	NC	NC
		EC_{50}/EC_{20}	1.2	–	–
Fe/Co magnetic fluid	Soil (t = 2 h)	EC_{20}	1982 (170.4–23,050)	NC (h.e. 80%)	NC (h.e. 86%
		EC_{50}	8755 (1858–41,250)	3483 (523.8–23,160)	2532 (242.6–26,420)
		EC_{50}/EC_{20}	4.4	–	–
	Soil (t = 30 days)	EC_{20}	6707 (1233–36,490)	NC (h.e. 70%)	NC (h.e. 74%)
		EC_{50}	14,920 (1699–13,100)	7190 (1463–34,560)	3592 (378.3–34,110)
		EC_{50}/EC_{20}	2.2	–	–
Gold nanorods 750 nm	Soil (t = 2 h)	EC_{20}	563.3 (26.58–11,940)	NC (h.e. 87%)	NC (h.e. 90%)
		EC_{50}	4068 (1047–15,800)	1468 (98.08–22,530)	1474 (62.28–34,870)
		EC_{50}/EC_{20}	7.2	–	–
	Soil (t = 30 days)	EC_{20}	843.6 (91.4–7818)	1065 (114.8–9887)	788 (43.52–14,270)
		EC_{50}	7581 (2411–23,830)	3793 (909.7–15,810)	2628 (449.9–15,350)
		EC_{50}/EC_{20}	8.99	3.56	3.33
TiO_2	Soil (t = 2 h)	EC_{20}	28,110 (NC)	17,580 (NC)	37,150 (2661–5,187,000)
		EC_{50}	63,500 (NC)	48,040 (NC)	57,000 (5156–630,200)
		EC_{50}/EC_{20}	2.3	2.7	1.5
	Soil (t = 30 days)	EC_{20}	12,930 (3513–47,590)	9697 (1429–65,780)	11,110 (1908–64,700)
		EC_{50}	66,820 (6720–664,500)	36,060 (2518–516,400)	37,660 (3278–432,700)
		EC_{50}/EC_{20}	5.2	3.7	3.4
$TiSiO_4$	Soil (t = 2 h)	EC_{20}	NC	NC	6311 (214–186,100)
		EC_{50}	NC (h.e. 17.2%)	NC (h.e. 15.7%)	NC (h.e. 21.3%)
		EC_{50}/EC_{20}	–	–	–
	Soil (t = 30 days)	EC_{20}	14,830 (1728–127,300)	14,380 (520.4–397,600)	NC
		EC_{50}	NC (h.e. 29.2%)	NC (h.e. 26.3%)	NC (h.e. 23.2%)
		EC_{50}/EC_{20}	–	–	–

NT – not toxic; NC – could not be computed; h.e. – highest effect.

3.2. Ames assay of soil elutriates

Elutriates obtained from soils spiked with Mo/NaO and SDS/DDBA have induced mutagenic effects on *S. typhimurium* (strain TA98). Despite the high induction factor (33.8) recorded for the lowest concentration of elutriate, obtained from the soil spiked with Mo/NaO (30 days) (Table 4), the genotoxic response was more consistent, in the assay with metabolic activation (Table 5). For the same elutriate the induction factors varied between 22 and 2.2, for the different concentrations tested even without a dose–effect relationship (Table 5). For elutriates of soil spiked with a suspension of SDS/DDBA, induction factors varied between 23.5 and 9.9, only for the lowest concentrations tested without S9, after 30 days of incubation (Table 4). No genotoxic effects were recorded when the S9 fraction was added to the assay (Table 5).

Elutriates from soils spiked with inorganic NPs were also genotoxic to *S. typhimurium*. The elutriate obtained from the soil spiked with the suspension of $TiSiO_4$ (30 days), displayed a genotoxic response in both bacteria strains. Significant dose–response curves were obtained for TA98 both with and without S9 (R^2 = 0.897,

Table 3
Effective concentrations (%) (95% CI, inside brackets) of the elutriates obtained after 2 h and 30 days, causing 20 and 50% inhibition in the bioluminescence (EC_{20} and EC_{50}, respectively), of *V. fischeri*.

Chemical	Test medium	Endpoint (%)	5 min	15 min	30 min
OECD soil + water	Elutriate (t = 2 h)	EC_{20}	NT	NT	NT
		EC_{50}	NT	NT	NT
		EC_{50}/EC_{20}	–	–	–
	Elutriate (t = 30 days)	EC_{20}	NT	NT	NT
		EC_{50}	NT	NT	NT
		EC_{50}/EC_{20}	–	–	–
OECD soil+ toluene	Elutriate (t = 2 h)	EC_{20}	NC (h.e. 18.8%)	NC (h.e. 21.2%)	NC (h.e. 23.2%)
		EC_{50}	NC (h.e. 18.8%)	NC (h.e. 21.2%)	NC (h.e. 23.2%)
		EC_{50}/EC_{20}	–	–	–
	Elutriate (t = 30 days)	EC_{20}	33.3% (10.2–110.8)	33.54 (7.5–150.5)	37.02 (26.06–51.5)
		EC_{50}	NC (h.e. 34.6%)	NC (h.e. 33.7%)	NC (h.e. 35.6%)
		EC_{50}/EC_{20}	–	–	–
Mo/NAO	Elutriate (t = 2 h)	EC_{20}	3.03 (1.19–7.72)	0.8 (0.51–1.24)	0.43 (0.3–0.62)
		EC_{50}	7 (4–5)	2 (1–3)	1 (NC)
		EC_{50}/EC_{20}	2.3	2.5	2.3
	Elutriate (t = 30 days)	EC_{20}	2.48 (1.14–5.37)	2.7 (1.24–5.89)	2.65 (1.34–5.26)
		EC_{50}	57.1 (22.8–142.7)	42 (17.8–99.2)	37 (18.1–75.8)
		EC_{50}/EC_{20}	23.0	15.5	13.9
SDS/DDBA	Elutriate (t = 2 h)	EC_{20}	0.27 (0.13–0.54)	0.19 (0.11–0.34)	0.2 (0.11–0.34)
		EC_{50}	0.93 (0.56–1.55)	0.73 (0.49–1.08)	0.62 (0.44–0.87)
		EC_{50}/EC_{20}	3.4	3.8	3.1
	Elutriate (t = 30 days)	EC_{20}	NT	NT	NT
		EC_{50}	NT	NT	NT
		EC_{50}/EC_{20}	–	–	–
Lumidot (CdSe/ZnS)	Elutriate (t = 2 h)	EC_{20}	33.8 (NC)	NT	NT
		EC_{50}	NT	NT	NT
		EC_{50}/EC_{20}	–	–	–
	Elutriate (t = 30 days)	EC_{20}	52.9 (14.6–191.6)	39.8 (7.7–205.4)	12.2 (2.5–59.1)
		EC_{50}	NT (h.e. 22%)	NT (h.e. 21%)	NT (h.e. 27%)
		EC_{50}/EC_{20}	–	–	–
Fe/Co magnetic fluid	Elutriate (t = 2 h)	EC_{20}	74.5 (NC)	35.2 (8.9–139.1)	30.3 (26.1–35.1)
		EC_{50}	NT	NT (h.e. 31%)	NT
		EC_{50}/EC_{20}	–	–	–
	Elutriate (t = 30 days)	EC_{20}	NC (h.e. 39%)	0.25 (NC)	0.36 (NC)
		EC_{50}	NC (h.e. 39%)	0.97 (NC)	1.14 (NC)
		EC_{50}/EC_{20}	–	–	–
Gold nanorods 750 nm	Elutriate (t = 2 h)	EC_{20}	4 (0.29–55.6)	NT	NT (h.e. 24%)
		EC_{50}	NT (h.e. 24%)	NT (h.e. 25%)	NT (h.e. 24%)
		EC_{50}/EC_{20}	–	–	–
	Elutriate (t = 30 days)	EC_{20}	NT	NT	NT
		EC_{50}	NT	NT	NT
		EC_{50}/EC_{20}	–	–	–
TiO_2	Elutriate (t = 2 h)	EC_{20}	NC	NC	NC
		EC_{50}	NC (h.e. 21.3%)	NC (h.e. 20.6%)	NC (h.e. 21.1%)
		EC_{50}/EC_{20}	–	–	–
	Elutriate (t = 30 days)	EC_{20}	16.72% (6.0–43.6)	28.3% (13.2–60.6)	23.9 (NC)
		EC_{50}	NT	NT	NT
		EC_{50}/EC_{20}	–	–	–
$TiSiO_4$	Elutriate (t = 2 h)	EC_{20}	2.4% (0.2–26.4)	3.8% (0.7–21.8)	4.5 (0.9–22.2)
		EC_{50}	17% (0.6–49.2)	NC (h.e. 40.1%)	NC (h.e. 37.6%)
		EC_{50}/EC_{20}	7.1	–	–
	Elutriate	EC_{20}	NC (h.e. 17.72%)	NC (h.e. 17.05%)	93.6 (NC)
		EC_{50}	NC (h.e. 17.72%)	NC (h.e. 17.05%)	NT
		EC_{50}/EC_{20}	–	–	–

NT – not toxic; NC – could not be computed; h.e. – highest effect.

$p = 0.000$ and $R^2 = 0.725$, $p = 0.004$, respectively) and for TA100 without S9 ($R^2 = 0.874$, $p = 0.000$). The metabolic activation, clearly reinforced the genotoxicity of this elutriate, since high induction factors were recorded (Tables 4–7), especially for TA98. Regarding elutriates obtained from soil spiked with the aqueous suspension of TiO_2, no genotoxicity was recorded for both strains (2 h and 30 days).

Elutriates from soils spiked with gold nanorods suspension and incubated 2 h were genotoxic for both *S. typhimurium* strains (Tables 4 and 6), without S9. After 30 days of incubation, the mutagenicity of the elutriate persisted for TA98 in the assay without S9 but also appeared when the liver fraction S9 was added (induction 2.4 and 3.5, respectively) for the lowest concentration tested (Tables 4 and 5). For the strain TA100 no mutagenicity was recorded (Table 7).

The elutriate obtained from soil spiked with the Fe/Co magnetic fluid, incubated for 30 days, was genotoxic to the TA98 strain (induction factors varying between 3.75 and 8.3), even without a significant dose–response curve (Table 4). This genotoxic response was not induced by toluene because elutriates obtained from soil spiked with a suspension of toluene (2 h and 30 days) were not genotoxic to both strains. The metabolic activation of the former

Table 4
Revertent colonies of TA98 strain (average ± STDEV) exposed to different concentrations of elutriates (1:4 m/v) obtained after 2 h and 30 days of incubation. Test performed without S9.

		100%	50%	25%	12.5%	6.25%
OECD soil + water	t = 2 h	8.3 ± 1.5	15.0 ± 2.0	16.2 ± 3.2	15.3 ± 2.5	10.3 ± 4.6
	t = 30 days	18.0 ± 3.0	17.3 ± 3.1	16.0 ± 4.2	18.7 ± 4.9	20.0 ± 8.2
OECD soil + toluene	t = 2 h	**33.67 ± 18.9**	10.67 ± 2.5	28.0 ± 10.1	9.0 ± 4.4	11.0 ± 2.6
	t = 30 days	11.66 ± 3.2	12.33 ± 5.5	13.3 ± 3.8	11.33 ± 0.58	9.3 ± 3.2
MO/NAO	t = 2 h	19.3 ± 11.7	13.0 ± 1.0	10.3 ± 2.1	15.0 ± 0.0	11.0 ± 2.0
	t = 30 days	11.7 ± 4.6	14.3 ± 4.0	16.7 ± 7.5	23.7 ± 6.5	**508.0 ± 79.2 (33.8)**
SDS/DDBA	t = 2 h	12.3 ± 4.2	16.3 ± 3.1	17.7 ± 1.5	16.7 ± 1.5	14.7 ± 1.5
	t = 30 days	19.3 ± 9.5	24.0 ± 8.0	18.0 ± 2.0	**353.0 ± 29.7 (23.5)**	**148.3 ± 8.0 (9.9)**
Lumidot (CdSe/ZnS)	t = 2 h	9.7 ± 2.3	14.3 ± 2.3	14.3 ± 0.6	12.7 ± 4.0	16.7 ± 2.1
	t = 30 days	16.7 ± 3.2	13.7 ± 5.0	15.0 ± 4.2	26.0 ± 11.8	14.0 ± 1.0
Fe/Co magnetic fluid	t = 2 h	11.7 ± 6.4	12.5 ± 0.7	13.0 ± 5.3	28.0 ± 5.3	10.5 ± 3.5
	t = 30 days	24.5 ± 9.2	**56.3 ± 2.3 (3.75)**	26.5 ± 16.3	**64.5 ± 14.8 (4.3)**	**124.7 ± 5.0 (8.3)**
Gold nanorods (750 nm)	t = 2 h	**28.3 ± 2.1 (2.1)**	**35.3 ± 12.1 (2.6)**	**32.7 ± 9.9 (2.4)**	26.3 ± 8.7	26.3 ± 4.9
	t = 30 days	16.0 ± 6.2	20.0 ± 8.9	20.7 ± 9.8	18.3 ± 4.2	**52.0 ± 15.6 (3.5)**
TiO_2	t = 2 h	14.0 ± 2.4	9.7 ± 2.1	10.0 ± 0.0	16.3 ± 4.0	11.33 ± 2.5
	t = 30 days	10.3 ± 1.2	11.67 ± 2.1	13.3 ± 3.5.1	14.3 ± 7.6	14.0 ± 6.1
$TiSiO_4$	t = 2 h	14.0 ± 1.7	13.0 ± 2.6	16.0 ± 7.2	17.0 ± 3.6	13.7 ± 2.5
	t = 30 days	**92.67 ± 9.9 (6.0)**	**47.0 ± 5.0 (3.1)**	**40.0 ± 16.0 (2.6)**	29.7 ± 2.5	18.7 ± 4.0
	t = 2 h	Negative control-wt	13.7 ± 3.1	Positive control	212.8 ± 58.7	
	t = 30 days		15.0 ± 7.1		157.0 ± 72.1	

Positive results in bold letter with corresponding induction factors calculated as the quotient between the average number of revertents obtained for that concentration and the average number of revertents on the negative control.

Table 5
Revertent colonies of TA98 strain (average ± STDEV) exposed to different concentrations of elutriates (1:4 m/v) obtained after 2 h and 30 days of incubation. Test performed with S9.

		100%	50%	25%	12.5%	6.25%
OECD soil + water	t = 2 h	25.0 ± 12.3	23.3 ± 3.5	18.0 ± 7.2	19.7 ± 7.0	15.3 ± 3.1
	t = 30 days	18.7 ± 2.5	20.0 ± 6.6	20.0 ± 5.0	22.0 ± 5.2	17.7 ± 4.7
OECD soil + toluene	t = 2 h	24.0 ± 2.8	31.0 ± 1.4	23.0 ± 7.1	16.0 ± 1.4	23.5 ± 6.4
	t = 30 days	39.7 ± 13.3	30.7 ± 3.8	19.7 ± 1.5	19.0 ± 1.5	21.3 ± 5.9
MO/NAO	t = 2 h	27.0 ± 4.6	36.7 ± 16.1	52.3 ± 20.1	36.3 ± 11.0	29.7 ± 12.3
	t = 30 days	**539.0 ± 41.0 (22.0)**	**193.5 ± 79.9 (7.8)**	**147.0 ± 15.6 (6.0)**	**53.0 ± 47.4 (2.3)**	**430.0 ± 23.6 (17.6)**
SDS/DDBA	t = 2 h	21.7 ± 8.5	25.3 ± 1.2	19.3 ± 5.9	22.7 ± 1.2	14.7 ± 1.5
	t = 30 days	21.0 ± 5.6	20.7 ± 3.5	36.3 ± 13.3	17.3 ± 2.3	13.7 ± 4.7
Lumidot (CdSe/ZnS)	t = 2 h	18.7 ± 5.7	17.3 ± 3.8	15.3 ± 6.0	27.7 ± 8.6	22.0 ± 2.6
	t = 30 days	27.0 ± 6.9	**54.0 ± 14.1 (2.2)**	25.5 ± 12.0	**59.5 ± 7.8 (2.4)**	31.7 ± 16.8
Fe/Co magnetic fluid	t = 2 h	34.7 ± 5.5	20.0 ± 5.6	20.0 ± 1.7	23.3 ± 2.1	16.3 ± 4.2
	t = 30 days	15.7 ± 3.2	NA	NA	13.7 ± 1.2	17.7 ± 3.8
Gold nanorods (750 nm)	t = 2 h	40.3 ± 7.2	34.0 ± 11.4	37.7 ± 2.5	37.0 ± 20.9	32.7 ± 6.8
	t = 30 days	21.0 ± 2.6	34.3 ± 13.6	50.0 ± 11.4	**57.0 ± 32.7 (2.3)**	24.3 ± 6.8
TiO_2	t = 2 h	29.0 ± 5.7	12.0 ± 9.9	25.5 ± 6.4	25.0 ± 5.7	27.5 ± 6.4
	t = 30 days	21.5.0 ± 9.9	26.5 ± 0.7	19.5 ± 20.5	28.0 ± 5.7	17.0 ± 0.0
$TiSiO_4$	t = 2 h	25.0 ± 2.8	21.0 ± 7.07	25.0 ± 11.3	26.5 ± 2.1	31.5 ± 3.5
	t = 30 days	**814.0 ± 87.7 (33.0)**	**702.0 ± 121.6 (28.6)**	**500.0 ± 141.4 (20.4)**	**162.5 ± 6.4 (6.6)**	21.0 ± 0.0
	t = 2 h	Negative control-wt	22.4 ± 9.3	Positive control		283.0 ± 54.9
	t = 30 days		24.5 ± 0.7			289.1 ± 157.0

Positive results in bold letter with corresponding induction factors calculated as the quotient between the average number of revertents obtained for that concentration and the average number of revertents on the negative control.

Table 6
Revertent colonies of TA100 strain (average ± STDEV) exposed to different concentrations of elutriates (1:4 m/v) obtained after 2 h and 30 days of incubation. Test performed without S9.

		100%	50%	25%	12.5%	6.25%
OECD soil + water	t = 2 h	178.0 ± 9.8	210.0 ± 20.0	205.0 ± 29.8	199.0 ± 22.6	199.0 ± 12.2
	t = 30 days	230.0 ± 30.2	226.3 ± 39.1	246.0 ± 11.1	207.0 ± 39.6	243.3 ± 11.0
OECD soil + toluene	t = 2 h	158.5 ± 16.3	150.5 ± 19.1	116.0 ± 18.4	150.0 ± 12.8	138.5 ± 4.9
	t = 30 days	132.7 ± 12.7	126.5 ± 20.5	121.5 ± 9.2	126.0 ± 14.1	136.0 ± 18.4
MO/NAO	t = 2 h	288.0 ± 83.9	211.7 ± 24.6	228.7 ± 29.7	199.0 ± 17.0	157.7 ± 27.3
	t = 30 days	238.7 ± 21.6	238.0 ± 29.1	240.7 ± 23.0	214.7 ± 19.6	218.0 ± 17.1
SDS/DDBA	t = 2 h	223.3 ± 15.9	237.3 ± 27.3	224.0 ± 5.2	205.7 ± 6.7	139.7 ± 15.0
	t = 30 days	254.0 ± 52.0	227.0 ± 37.7	229.0 ± 22.6	244.3 ± 19.3	219.7 ± 22.9
Lumidot (CdSe/ZnS)	t = 2 h	121.3 ± 11.0	124.7 ± 7.0	168.0 ± 8.5	159.7 ± 2.1	145.7 ± 19.1
	t = 30 days	218.0 ± 11.1	179.7 ± 28.7	207.0 ± 21.9	201.7 ± 4.0	184.0 ± 26.0
Fe/Co magnetic fluid	t = 2 h	218.3 ± 21.2	204.7 ± 22.0	214.7 ± 33.7	190.3 ± 41.5	246.3 ± 38.1
	t = 30 days	222.7 ± 31.0	219.7 ± 12.3	198.3 ± 23.2	216.3 ± 7.2	201.3 ± 39.0
Gold nanorods (750 nm)	t = 2 h	333.5 ± 12.3	237.3 ± 37.6	348.3 ± 42.1	305.7 ± 53.4	245.0 ± 43.3
	t = 30 days	175.7 ± 39.1	204.7 ± 28.0	208.7 ± 28.9	183.3 ± 5.7	176.7 ± 22.2
TiO_2	t = 2 h	144.3 ± 10.1	152.7 ± 45.3	117.3 ± 5.5	113.7 ± 2.5	111.7 ± 14.5
	t = 30 days	154.0 ± 25.5	103.3 ± 25.3	115.0 ± 8.5	123.7 ± 24.7	131.7 ± 29.1
$TiSiO_4$	t = 2 h	115.0 ± 21.2	141.0 ± 26.9	92.5 ± 21.9	102.5 ± 13.4	95.5 ± 6.4
	t = 30 days	**375.0 ± 35.4 (2.8)**	**288.0 ± 11.3 (2.1)**	200.5 ± 84.1	147.0 ± 11.3	118.5 ± 3.5
	t = 2 h	Negative control-wt	175.7 ± 23.7	Positive control	589.3 ± 21.9	
	t = 30 days		136.0 ± 14.0		410.3 ± 67.2	

Positive results in bold letter with corresponding induction factors calculated as the quotient between the average number of revertents obtained for that concentration and the average number of revertents on the negative control.

Table 7
Revertent colonies of TA100 strain (average ± STDEV) exposed to different concentrations of elutriates (1:4 m/v) obtained after 2 h and 30 days of incubation. Test performed with S9.

		100%	50%	25%	12.5%	6.25%
OECD soil + water	t = 2 h	297.7 ± 35.6	311.3 ± 42.0	316.7 ± 58.7	272.3 ± 45.9	283.3 ± 48.0
	t = 30 days	187.7 ± 20.2	163.0 ± 13.0	168.0 ± 8.2	166.3 ± 5.5	173.5 ± 16.3
OECD soil + toluene	t = 2 h	169.5.0 ± 16.3	194.5 ± 3.5	170.5 ± 37.5	172.5 ± 4.9	127.5 ± 84.0
	t = 30 days	204.5 ± 13.4	180.0 ± 2.8	177.5 ± 19.1	193.0 ± 19.8	181.5 ± 44.5
MO/NAO	t = 2 h	242.0 ± 47.5	281.0 ± 20.0	262.0 ± 30.8	298.7 ± 74.6	275.3 ± 98.2
	t = 30 days	175.7 ± 26.0	170.0 ± 0.0	296.0 ± 13.0	229.5 ± 91.2	172.3 ± 32.5
SDS/DDBA	t = 2 h	150 ± 14.7	141.3 ± 11.6	154.0 ± 21.9	174.7 ± 21.2	152.0 ± 29.9
	t = 30 days	157.7 ± 1.7	130.7 ± 29.9	143.3 ± 17.9	120.3 ± 1.5	122.3 ± 17.6
Lumidot (CdSe/ZnS)	t = 2 h	256.7 ± 13.3	295.0 ± 79.5	210.2 ± 34.5	285.7 ± 46.6	246.3 ± 10.0
	t = 30 days	189.0 ± 84.0	188.0 ± 23.3	222.7 ± 66.7	**386.0 ± 62.2 (2.8)**	**716.0 ± 248.9 (5.1)**
Fe/Co magnetic fluid	t = 2 h	167.3 ± 5.5	159.0 ± 4.6	164.0 ± 13.5	142.7 ± 12.9	155.3 ± 28.7
	t = 30 days	167.3 ± 5.5	159.0 ± 4.6	164.0 ± 13.5	142.7 ± 12.9	155.3 ± 28.7
Gold nanorods (750 nm)	t = 2 h	257.7 ± 16.5	209.7 ± 43.8	216.7 ± 10.7	224.0 ± 6.1	212.3 ± 32.9
	t = 30 days	189.7 ± 28.1	224.0 ± 23.5	148.0 ± 29.5	172.3 ± 3.8	172.3 ± 32.6
TiO_2	t = 2 h	164.5 ± 36.1	120.5.0 ± 21.9	173.5 ± 23.3	156.0 ± 5.6	177.0 ± 0.0
	t = 30 days	169.5 ± 6.4	203.5 ± 3.5	184.5 ± 27.6	210.5 ± 0.7	251.0 ± 0.0
$TiSiO_4$	t = 2 h	152.5 ± 4.9	266.3 ± 40.0	270.3 ± 52.6	282.3 ± 68.3	293.3 ± 44.5
	t = 30 days	**616.0 ± 39.6 (4.4)**	**752 ± 11.3 (5.4)**	**746.0 ± 178.1 (5.3)**	**490.0 ± 121.6 (3.5)**	186.0 ± 0.0
	t = 2 h	Negative control-wt	261.3 ± 20.9	Positive control		608.0 ± 33.4
	t = 30 days		140.3 ± 25.1			446.6 ± 63.3

Positive results in bold letter with corresponding induction factors calculated as the quotient between the average number of revertents obtained for that concentration and the average number of revertents on the negative control.

elutriate, has made it toxic to the TA98 strain, since a significant reduction in the number of revertent colonies was recorded, on plates, when compared with the negative control (Dunnett's test: $p < 0.05$). Such toxicity was probably responsible for the apparent absence of a genotoxic response (Table 5). In opposition elutriates of OECD soils spiked with an aqueous suspension of QDs (30 days of incubation) were genotoxic to both strains, but only in the assays with S9 (Tables 5 and 7).

The strain TA100 of *S. typhimurium* was particular sensitive to elutriates of soils spiked with aqueous suspensions of SDS/DDBA, $TiSiO_4$, Fe/CO, and TiO_2. When these elutriates were obtained, after 2 h of incubation, they were toxic to bacteria, giving rise to a

significant reduction in the number of revertents in the plates (Dunnett's test: $p < 0.05$). Such toxicity was particularly evident for assays with S9.

4. Discussion

Size and surface charge of NPs in suspension were already considered, as the most relevant properties to infer about their mobility in the soil [38]. In this context, DLS has been used and recommended as a useful technique for real-time evaluation of these properties in aqueous suspensions [39] and it has been applied in several studies focusing both the fate and toxicity of NPs [40–42].

Despite concerns about keeping NPs in their nanosize, when dispersed in suspensions used for toxicity evaluation, in this study authors have decided to prepare aqueous suspensions of NPs, following a simple methodology (magnetic stirring), to guarantee their ecological relevance. This procedure was in agreement, with recommendations from Tiede et al. [1] who have mentioned that the test of NPs for ERA purposes should not be made with manipulated exposures, since their occurrence in natural systems is highly unlike.

Despite their aggregation, all the NPs were able to change the quality of the spiked soil, and corresponding elutriates, making them toxic and/or genotoxic to *V. fischeri* and *S. typhimurium* (TA98 and TA100). Such observation suggests size is not the only property responsible for the toxicity and genotoxicity of these NPs.

The OECD soils spiked with aqueous suspensions of SDS/DDBA, MoNaO, gold nanorods or of Fe/Co magnetic fluid were very toxic to *V. fischeri*, especially after 2 h of incubation. The lowest EC_{50} values obtained for soils spiked with aqueous suspensions of NPs, when compared to the EC_{50} value obtained for soil with distilled water, suggest that these suspensions have contributed for increasing even more soil toxicity. In fact aqueous suspensions of the above mentioned NPs have proved to be very toxic to *V. fischeri* when tested directly through a Microtox Basic test (Lopes et al. unpublished data).

The high toxicity recorded after 2 h of soil incubation suggests that this period was likely not sufficient for the establishment of equilibrium conditions between NPs and soil components, rendering them available to exert toxic effects. In fact some elutriates obtained after 2 h of incubation (soils spiked with SDS/DDBA vesicles, $TiSiO_4$, and TiO_2) were also toxic to the strain TA100 of *S. typhimurium*, inhibiting the growth of bacteria colonies. Further the inexistence of equilibrium conditions and the likely great mobility of NPs, may also explain why elutriates obtained from soils spiked with the vesicles of SDS/DDBA, Mo/NaO or with Fe/Co magnetic fluid were particularly toxic to *V. fischeri* when they were obtained after 2 h of incubation.

The greater stability of the negatively charged SDS/DDBA vesicles (zeta potential < −30 mV), the low PDI value of the suspension and their nano size in aqueous suspension, was probably responsible by the maintenance of soil toxicity, between 2 h and 30 days of soil incubation. However, the hypothetical degradation of these organic NPs, in more simple and toxic forms, by the soil microbial community, cannot be discarded, as well. In opposition, the positively charged gold nanorods, which maintained their nano size in aqueous suspension, may have adsorbed strongly to organic matter, becoming unavailable to exert toxic effects and to be eluted from soil. This may explain the non toxicity of elutriates obtained from soil samples spiked with an aqueous suspension of this NP, throughout the incubation period.

The negatively charged and stable Mo/NaO and SDS/DDBA vesicles (ζ potential < −30 mV) may have been eluted in great quantity, especially after 2 h of soil incubation (before reaching an equilibrium). Elutriates obtained from soil spiked with Fe/Co magnetic fluid were also very toxic, especially after 30 days of incubation. The degradation of the capping agents with ageing, may also have contributed for the release of Co, and degradation of the quality of soils spiked with these NPs and of corresponding elutriates. The same could have happened with the core shell particles CdSe/ZnS QDs, for which a long period of soil incubation may have contributed for the degradation of the shell, and the release of Cd^{2+}. In fact, QDs have been found to degrade under photolytic and oxidative conditions [43]. Kloepfer et al. [44] recorded evidences of metal damage in *Bacillus subtilis* cells incubated with core CdSe QDs, in opposition to cells incubated with the core shell QDs (CdSe/ZnS). However, in our study it is not possible to distinguish the role of QDs from that of the soil components and of toluene, in the toxicity of soil and elutriates to *V. fischeri*.

The results obtained for *V. fischeri* suggest that ageing acts differently, maintaining/decreasing (soils spiked with suspensions of organic NPs) or increasing (soils spiked with suspensions of gold nanorods and Fe/Co magnetic fluid) the toxicity of soils. As Lecoanet et al. [45] and French et al. [46] pointed out numerous factors may combine to increase NPs mobility. According to these authors, polyelectrolytes like humic and fulvic acids, which are naturally found in soils, might adsorb to NPs, reducing the reactivity of their surfaces through steric and electrostatic stabilization and increasing their mobility to the aqueous phase. Hence the existence of a great percentage of organic matter *per si*, is not a guarantee of adsorption and reduced bioavailability of NPs. Moreover, different modes of action of NPs, determined by their elemental composition probably had a role in the different toxic and genotoxic responses.

The TA98 strain of *S. typhimurium* showed to be more sensitive to elutriates of soils spiked with both suspensions of organic and inorganic NPs, than TA100. Such observations suggest that at least soils spiked with suspensions of SDS/DDBA and MoNaO, of gold nanorods, of QDs and of Fe/Co were able to revert frameshift type mutations [35]. The metabolic activation by the liver fraction S9 was also able to modulate the mutagenicity of the elutriates, eliminating the genotoxicity of those obtained from soils spiked with SDS/DDBA vesicles and with gold nanorods, to the strain TA98, but increasing the genotoxicity of elutriates from soil spiked with CdSe/ZnS QDs, for the same strain. Only elutriates of soils spiked with gold nanorods, CdSe/ZnS QDs and $TiSiO_4$ induced mutations in both strains, suggesting more diversified mechanisms of toxicity. Once again the fraction S9 has acted differently, eliminating or stimulating the genotoxic activity of the above mentioned elutriates to TA100. The fraction S9, conjugated with ageing, may have promoted the degradation of the shell of CdSe/ZnS QDs, with release of Cd^{2+}, and subsequent mutagenicity on *S. typhimurium*. Such occurrence may explain the positive results recorded for both strains, after 30 days of soil incubation and in the assay with S9. Although Codina et al. [47] did not observe positive responses for Cd, in the Ames assay, the Mutatox assay has confirmed the genotoxicity of this metal to bacteria.

The TA100 was particularly sensitive to soil elutriates. The same observation was reported by Pan et al. [48] when testing suspensions of TiO_2.

The antibacterial activity of TiO_2 has been reported by other authors [e.g. 50], sometimes induced by photoactivation [e.g. 51], but also depending on the strain tested [48,49]. The non mutagenicity of elutriates of soil spiked with a suspension of TiO_2 to *S. typhimurium* was in agreement with negative responses recorded by other authors [49,51, Lopes et al., unpublished data]. In our study the negative response recorded may have resulted from the large size and relative stability (zeta potential < +30 mV) of TiO_2 aggregates in aqueous suspension. In fact other authors have reported the genotoxicity of nano TiO_2, with small particle sizes, and with different ratios of anatase/rutile, evaluated through other test systems (e.g. comet assay and micronucleus) [e.g. 51–54].

To the best of our knowledge, no ecotoxicological data exists for $TiSiO_4$ NPs. In this study, aqueous suspensions of $TiSiO_4$, were responsible for the mutagenic potential of soil samples for both TA98 and TA100 strains, after 30 days of soil incubation, despite the great size of aggregates formed. Once again the instability of $TiSiO_4$ NPs after 2 h of soil incubation may have been responsible by their availability to yield toxic effects on *S. typhimurium* (TA100). In opposition and after 30 days, aggregation/disaggregation processes promoted by soil organic matter may explain the mutagenic effects of soil samples. Baalousha et al. [22] has studied this phenomena of NPs disaggregation promoted by organic matter, for the aquatic compartment, also suggesting their importance in the terrestrial compartment.

The use of the standard artificial soil OECD [31] increases the reproducibility and the comparability of the assays evaluating the fate and toxicity of different NPs. These aspects are particularly relevant, especially when few ecotoxicological information is available.

5. Conclusion

This study provides further evidences about the potential of NPs for compromising soil quality as demonstrated by the increased toxicity (to *V. fischeri* and *S. typhimurium*) and genotoxicity (to *S. typhimurium*), of OECD soils, spiked with aqueous suspensions of the NPs. In general, toxicity and genotoxicity was higher 2 h after soil contamination suggesting that this period was not sufficient for the stabilization of NPs in the soil matrix, which were likely more available to exert toxic effects. Ageing may have either contributed for the establishment of strong interactions of nanoparticles with the soil components or, in opposition, may have promoted the degradation of organic shells (Fe/Co magnetic fluid) with release of core elements and increased toxicity. For some NPs, the toxicity and genotoxicity of soil samples was recorded despite aggregation has occurred in the spiking solution.

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