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Influence of environmental media on carbon nanotubes and graphene nanoplatelets towards bacterial toxicity

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Abstract: Functional carbon-based nanomaterials have become important due to their unique combinations of chemical and physical properties, and also because of the increasing research efforts in various fields. A significant gap in nanotechnology is the disregarding of physicochemical transformation under real conditions for the examination and comparison on the effect of carbon based nanomaterials. In this study, the behavior of some carbon based nanomaterials (multi-walled carbon nanotubes and graphene nanoplatelets) in environmental media (sea water, soil, and airborne fine particulate) were evaluated by using the influence on nanomaterial physicochemical properties (size, zeta potential, surface chemistry, morphology and sedimentation) and on the toxicity of bacterium (gram positive and gram negative bacteria) to contribute to their environmental hazard and risk assessment on the environment. The bacteria were exposed to the carbon based nanomaterials, and cultivated on nutrient agar plates including each environmental media, and then counted for the colony forming units. The physicochemical properties of the carbon based nanomaterials dispersed in these environmental media were also investigated. Our results indicated that the toxicity depended on the type of environmental media and their concentration, and the physicochemical properties of the carbon based nanomaterials changed when compared to the results obtained in controlled conditions.

Introduction

Functional carbon-based nanomaterials (CBNs) such as graphane nanoplatelets (GNPs) and carbon nanotubes (CNTs) have become important due to their unique combinations of chemical and physical properties (i.e., thermal and electrical conductivity, high mechanical strength, and optical properties), and also because extensive research efforts are being made to utilize these materials for various industrial applications (biomedical, environmental, and energy etc.) (Perez et al. 2009, Figarol et al. 2015, Kang et al. 2009, Oberdorst et al. 2006, Aschberger et al. 2010, Chatterjee et al. 2014a, Allegria et al. 2016). CBNs are the thinnest possible configuration of carbon molecules, and are a basic building block for other graphitic materials such as graphene, graphite, large fullerenes, and CNTs. The two active parts: surfaces and edges, facilitate the graphene attaching to the biological molecules and adhering to the cells (Yang et al. 2013, Yang et al. 2012, Zhao et al. 2014, Akhavan and Ghaderi 2010). Due to their increasing production and application, CBNs are released into the various environmental media (air, soil, and water systems), and this issue causes significant concerns.

Environmental media refer to the abiotic components of the natural environment, namely, air, water and soil. Their parameters such as pH, electrolytes and organic compounds etc. are known to affect the properties of nanomaterials. Recent studies have also indicated the importance of environmental media on nanomaterials' behavior and toxicity (Montegner et al. 2017, Lalwani et al. 2016, Jastrzebska et al. 2012, Park et al. 2013, Djurisic et al. 2014, Aruoja et al. 2015, Maurer-Jones et al. 2013, Joo and Zhao 2017, Simon-Deckers et al. 2009). Nevertheless, previous studies mostly focused on aqueous systems (seawater and wastewater, etc.) and metal oxide nanoparticles. A relatively small amount of information has been generated about CBNs and their behavior in different environmental media. Thus, in order to make realistic correlations with the environment, the interaction and transformation of the CBNs should be investigated on the abiotic components in the natural environment.

Evaluating nanomaterial activity against bacteria is an important step towards understanding of the environmental impact. These model organisms are responsive and sensitive to various damaging factors, and their physiological appearance allows for the understanding of the toxicity mechanisms. The toxicity of CBNs against bacteria has been studied in a lot of research, and the results in many publications indicate that CBNs exert non toxicity to measurable toxicity both in

vitro and *vivo* studies in various types of microorganisms, also the behavior of CBNs in environmental media is mostly disregarded in ecotoxicity studies (Montegner et al. 2017, Yang et al. 2012, Yang et al. 2013). Multiwalled carbon nanotubes (MWCNTs) seem to be less toxic to bacteria when compared to single walled carbon nanotubes due to less interaction between the bacteria and MWCNT resulting in the higher rigidity and probably lesser van der Waal's forces on the MWCNT surface (Aschberger et al. 2010, Chatterjee et al. 2014, Allegria et al. 2016, Khalid et al. 2016). Contrary to the toxicity of the MWCNTs studied in different microorganisms, GBNs have been investigated in a limited amount of studies using bacteria. The bacterial activity of the graphene and its derivatives were investigated in terms of *Escherichia coli*, *Staphylococcus aureus*, and *Shewanella* strains either in a controlled (laboratory) condition or simulated condition. Unfortunately, other bacteria strains important for the environment have yet to be investigated (Liu et al. 2011, Bykkam et al. 2013, Wang et al. 2011, Combarros et al. 2016, Efremova et al. 2015, Chatterjee et al. 2014, Krishnamoorthy et al. 2012, Seabra et al. 2014, Guo and Mei 2014, Singh 2016, Jastrzebska et al. 2012, Akhavan and Ghaderi 2010, Akhavan and Ghaderi 2012, Bai et al. 2012, Di Sotto et al. 2009, Simon-Deckers et al. 2009, Kang et al. 2008, Kang et al. 2009, Zardini et al. 2012, Zardini et al. 2014).

Moreover, some necessary information is missing or limited; the behavior of CBNs in different environmental media (real conditions), the toxicity in these media towards organisms, and their comparisons are lacking in this field to evaluate the environmental hazard of the CBNs. Taking into account all these considerations, the aim of the present study is to better understand the environmental impacts of CBNs, to analyze the effect of media on the physicochemical properties (particle size, surface charge, surface chemistry, morphology and sedimentation) of the CBNs. The concentration dependent inhibition of the CBNs was investigated under various concentrations of different environmental media on gram--negative and gram-positive bacteria.

Materials and methods

Reagents

GNPs were obtained from Nanografi (Ankara, Turkey). MWCNTs (Ctube100) were purchased from Cnt Co. Ltd., South Korea, (http://www.carbonnanotube.biz). All chemicals were of analytical grade (Merck, Germany; Fluka, Switzerland). Nutrient agar was obtained from Merck (Merck 1.05450.0500).

The model organisms used in this study were gram--negative bacteria (*Escherichia coli* (*E. coli)* ATCC 25922, and *Pseudomonas aeruginosa* (*P. aeruginosa)* ATCC 27853) as well as gram-positive bacteria (*Bacillus subtilis* (*B. subtilis)* ATCC 6633, and *Staphylococcus aureus* (*S. aureus)* 27853).

Environmental media information

To find the environmental effect on CBNs, soil, airborne fine particulates ($PM_{2.5}$) and sea-water were used as environmental media. The soil, $PM_{2.5}$ and sea-water samples were taken in Istanbul and their extracts were prepared in two concentrations in ultra-pure water to reflect the typical concentrations of low and high levels according to the available limit values or standard sample preparation procedures. Some sampling information is given in Table 1. Also, some chemical properties of the selected environmental media are shown in Table 2 and their analysis procedures are given in Supplementary Table 1.

The $PM_{2.5}$ samples were collected using AirFlow HVS (Analitica Strumenti, Pesaro, Italy) high-volume aerosol samplers equipped with $PM_{2.5}$ head in Maslak, Istanbul. The $PM_{2.5}$ extracts were prepared in concentration of 2.5 µg/L and 25 µg/L. To prepare the extracts, $PM_{2.5}$ collected on a filter was sonicated in ultra-pure water for 20 min. A high concentration was selected to simulate EU air regulation limit for $PM_{2.5}$, for which the limit concentration of $PM_{2.5}$ in air is 25 µg/L (Szigeti et al 2013, Baysal et al. 2017). The soil extracts were prepared in concentration of 0.1 g/mL and 1.0 g/mL in ultra pure water (1.0 g/mL is selected from standard procedure for the major ion determination and reflects high concentration). The soil samples taken from Maslak, Istanbul, were weighted and mixed in ultra-pure water for 20 min. Sea water samples applied at two concentrations; i) sea water without any dilution (referred as high concentration, ii) 1:10 diluted with ultra-pure water (referred as low concentration)

Preperation and characterization of carbon based nanomaterials

The tested CBNs were mixed with the sea-water, soil, and $PM_{2.5}$ extracts (2.5 mg, 5.0 mg, and 25 mg NMs in one liter extract) for 24 hours and then CBNs were dried until the full evaporation of water. All measurements were repeated at least five times. To reflect the control conditions, CBNs were treated with ultra-pure water using the same procedure and used as control. The concentration of CBNs selected as 2.5 mg/L,

Table 1. Some information about environmental samples and sampling

Environmental sample type	Applied concentration of environmental samples		Sampling information	Extraction procedure
Soil	Low concentration (L): 0.1 mg/L	High concentration (H): 1.0 mg/L	collected in Istanbul – Turkey	mixing in ultra pure water during 20 min (0.9% NaCl)
$PM_{2.5}$	Low concentration (L): 2.5 µg/L	High concentration (H): 25 µg/L	collected in Istanbul – Turkey by PM2.5 high volume air sampler on a quartz filter	mixing in ultra pure water during 20 min (0.9% NaCl)
Sea water	Low concentration (L): 1:10 diluted	High concentration (H): direct	collected in Bosphours, Istanbul – Turkey	–

Table 2. Chemical characterization of the environmental media

	Chemical characterization of the environmental media				
Media	SO_4^{2-},	NO_3^-,	NH_4^+,	Cl^-,	pH
Low concentration of soil	0.54±0.74 mg/L	ND	ND	22.7	6.5
High concentration of soil	4.96±0.52 mg/L	0.55±0.09 mg/L	0.26±0.11 mg/L	230±346 mg/L	6.5
Low concentration of $PM_{2.5}$	0.25±0.07 µg/L	0.09±0.03 µg/L	0.08±0.03 µg/L	0.02±0.01 µg/L	7.0
High concentration of $PM_{2.5}$	2.66±1.12 µg/L	1.06±0.65 µg/L	0.84±0.23 µg/L	0.29±0.11 µg/L	7.0
Low concentration of sea water	278.4 mg/L	ND	3.72 mg/L	0.59 mg/L	8.0
High concentration of sea water	2843.0 mg/L	ND	38.90 mg/L	6.01 mg/L	8.0

Supplementary Table 1. Some chemical analysis of media, and related information about the analysis

Parameter	Method	Instrument	Reference
SO_4^{2-}	Turbidimetric as barium sulfate (375.4): Sulfate ion is converted to a barium sulfate suspension under controlled conditions. The resulting turbidity is determined spectrophotometrically at 420 nm.	UV-VIS spectrometry (Biochrom Libra S70 spectrophotometer)	Water and Environmental Analysis 2010; Environmental Monitoring Systems Laboratory (EMSL) 1983
NO_3^-	Sulfanilamide/ethylenediamine with Cd reduction (353.3): The nitrite (that originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is measured spectrophoto-metrically at 540 nm	UV-VIS spectrometry (Biochrom Libra S70 spectrophotometer)	Water and Environmental Analysis. Perkin Elmer, 2010; Environmental Monitoring Systems Laboratory (EMSL) 1983; American Public Health Association 1992.
NH_4^+	Nesselerization (APHA 4500): The sample is buffered at a pH of 9.5 with a borate in order to decrease hydrolysis of cyanates and organic nitrogen compounds and is then distilled into a solution of boric acid. The ammonia in the distillate is determined colorimetrically by Nesslerization at 425.0 nm by spectrometrically.	UV-VIS spectrometry (Biochrom Libra S70 spectrophotometer)	Water and Environmental Analysis. Perkin Elmer, 2010; Environmental Monitoring Systems Laboratory (EMSL) 1983; American Public Health Association 1992
Cl^-	Chromatographic separations were performed at 30°C with a Dionex IonPac AS20 analytical column (2×250 mm). In addition, guard column and cartridge using ultra-pure (UP) water obtained from Dionex. The gradient programme: 10 mM of KOH for 6 min; linear increase of the KOH concentration from 10 mM to 25 mM for 15 min; 25 mM of KOH for 4 min; linear increase of the KOH concentration from 25 mM to 40 mM for 5 min; 40 mM of KOH for 5 min; linear decrease of the KOH concentration from 40 mM to 10 mM for 2 min. A 75 µL-aliquot of the sample/standard solution was loaded into the eluent stream. Flow rate of 2.5 mL/min.	Ion chromatograpy (Dionex ICS-3000)	Szigeti et al. 2013

5.0 mg/L, and 25 mg/L due to the applied concentrations are in the range of 0–4000 mg/L to characterize and form the bacteria model system (Ou et al. 2016, Montagner et al. 2017).

The characterization of the CBNs is performed to investigate their particle size, zeta potential, surface chemistry, morphology and sedimentation.

Surface chemistry was investigated using Fourier-transform infrared (FTIR) spectrometry (Bruker). The FTIR analysis was acquired in the range of 4000 to 500 cm^{-1} to investigate the environmental media effect on surface chemistry of control and treated CBNs.

The morphology of CBNs was determined using a Quanta FEG250 (Thermo Scientific, Hillsboro, OR, USA) scanning electron microscope (SEM).

Particle size and zeta potentials of the CBNs in suspensions were measured via dynamic light scattering (DLS) using Zeta sizer Nano ZS instruments (Malvern, UK) at 25°C at 173° scattering angle with 4 mW He-Ne laser. A 1.0 mg of control and treated CBNs were suspended in 1 mL ultra-pure water, and then sonicated for 5 min. If it is necessary, the CBNs were diluted in 100 µg/mL concentration and placed in Standard Malvern zeta potential disposable capillary cells and

polystyrene cuvettes for zeta potential and size measurements, respectively.

For the sedimentation experiments, CBNs dispersions were prepared using the similar protocol explained in DLS experiments. The sedimentation rate (A/A_0) was determined by monitoring the optical absorbance (at 660 nm) as a function of time, during a time interval of 0 and 24 h, which indicates A_0 and A, by ultraviolet–visible (UV–VIS) spectrophotometry (Libra S70 UV-VIS spectrophotometer, BioChrom, Cambridge, UK). All measurements were made at 25°C in square cuvettes with 1 cm light path, the center of the light beam striking the cuvette 1.5 cm above its bottom.

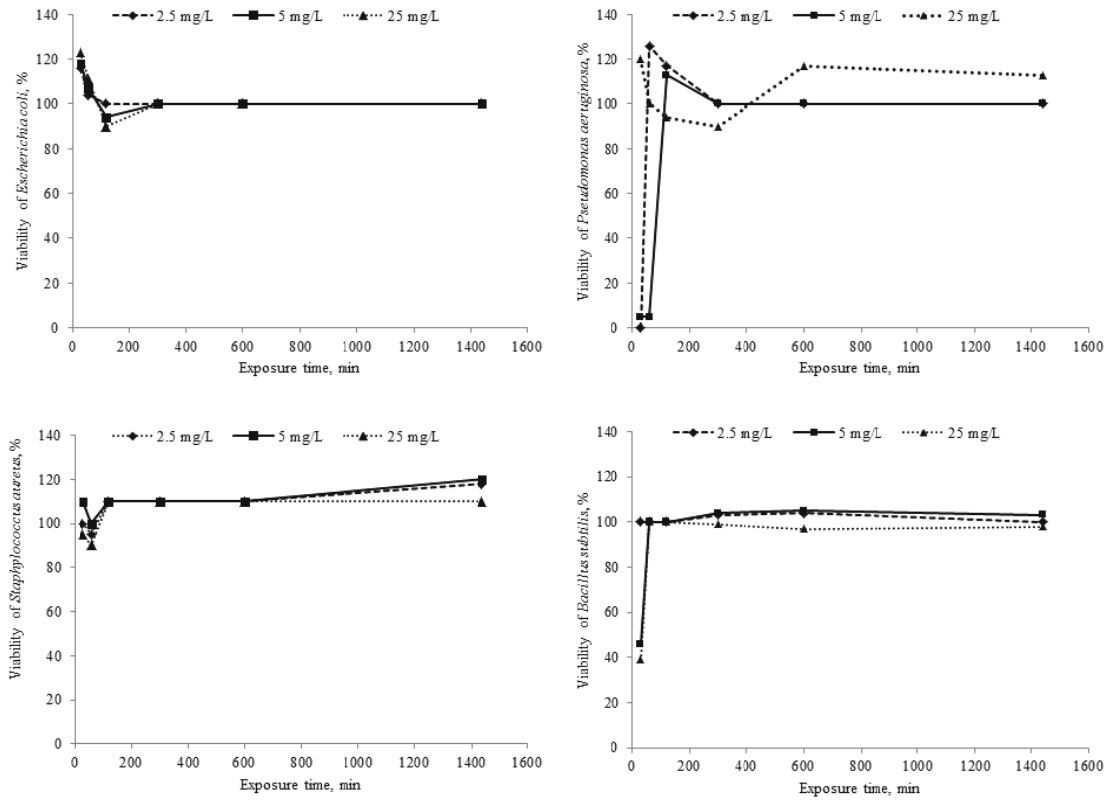
Growth inhibition assay

To form the bacteria model system, firstly the exposure or contact time was investigated between CBNs and selected bacteria (*E. coli, P. aeruginosa, S. aureus*, *B. subtilis*) in controlled conditions. For this purpose, the toxicity assessment was performed according to Jiang, Mashayekhi, and Xing (2009) and Baek and An (2011). The toxicity tests were conducted in Petri dishes (90 mm×18 mm). Each dish contained nutrient agar culture medium with a specific CBNs concentration (2.5, 5.0 and 25 mg/L). Approximately 15 mL of a 2% agar solution was poured into a test unit and immediately hardened in a freezer to avoid the possible precipitation of NPs (Baek and An 2011). Cultures of each of the microorganisms were prepared at 37°C in darkness overnight using nutrient broth, and 100 µL was used to inoculate the agar Petri dishes. The test units were then placed in an incubator (Thermo-Herathem IGS 100 Incubator, Thermo Fisher Scientific, Langenselbold, Germany) at a controlled temperature of 37°C. Each agar concentration (e.g., treatment) was prepared in three replicates. After a test incubation period (30 min, 60 min (1 h), 120 min (2 h), 300 min (5 h), 600 min (10 h), 1440 min (24h)), colony forming units (CFU) were counted in each test unit using a stereoscope. Agar medium without CBNs was employed as a control (No) in each exposure time. N is the colony forming units (CFUs) on the solid nutrient agar medium with CBNs in selected concentration. Viability rate was calculated as%=(N/No)*100. The results are show in Supplementary data (Supplementary Fig. 1 and Fig. 2). Non-toxicity was observed for the tested CBNs and 24 h and a non-toxic exposure time of CBNs was chosen for further analysis to investigate environmental media effect.

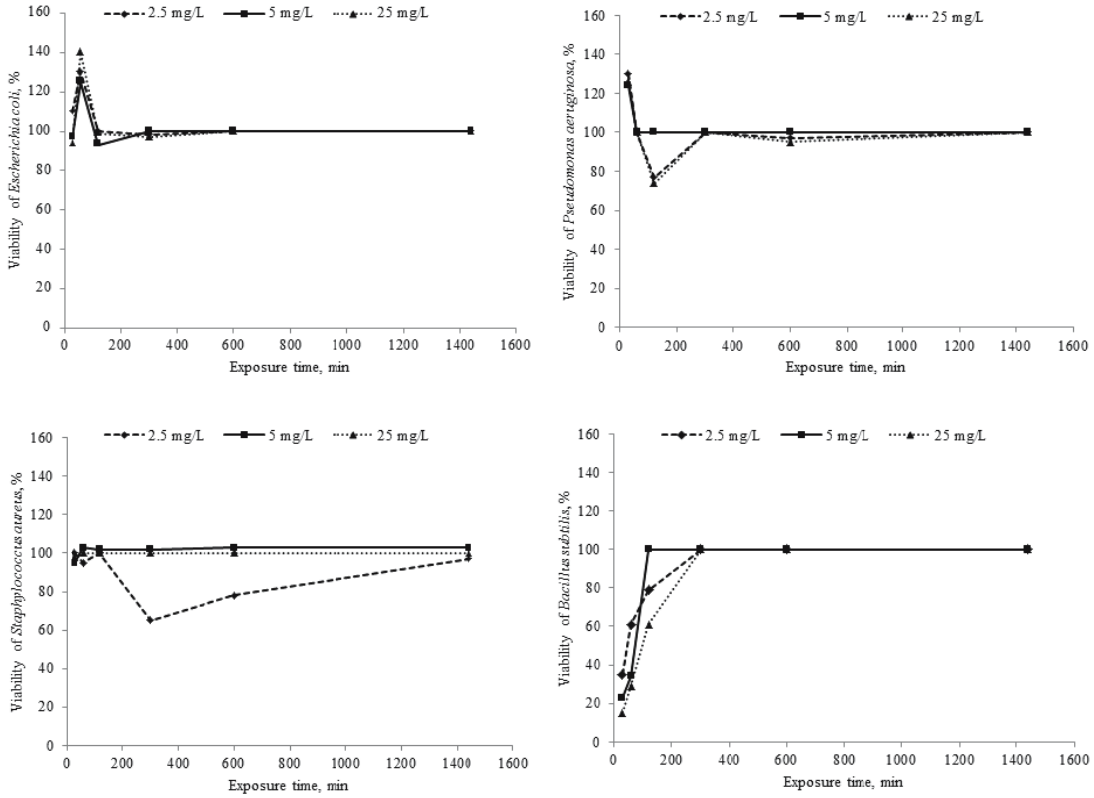
To investigate the effect of sea-water, soil, and $PM_{2.5}$ as an environmental media on CBNs by bacteria, the above-mentioned procedure was used and the 2% agar solution was prepared by each environmental media extract. For the control (No), without CBNs of the 2% agar solution prepared by each environmental media extracts was used. Each concentration was prepared in five replicates.

The susceptibility percentage was calculated in No and N, which:

No: growth medium prepared with environmental media (sea-water, soil, and $PM_{2.5}$ extracts) without CBNs (incubation time 24 h).



Supplementary. Fig. 1. Infuluence of exposure time on gram negative (Escherichia coli (a) and Pseudomonas aeruginosa(b)) and gram positive (Staphylococcus aureus (c) and Bacillus subtilis (d)) bacteria with multiwalled carbon nanotubes on bacteria viability. Percentage of viability of gram positive and gram negative bacteria due to CBNs exposure at different doses (2.5, 5.0, and 25 mg/L) for various exposure time (N=3)



Supplementary. Fig. 2. Supplementary. Fig. 2. Infuluence of exposure time gram negative (Escherichia coli (a) and Pseudomonas aeruginosa (b)) and gram positive (Staphylococcus aureus (c) and Bacillus subtilis (d)) bacteria with graphene nanoplatelets on bacteria viability. Percentage of viability of gram positive and gram negative bacteria due to CBNs exposure at different doses (2.5, 5.0, and 25 mg/L) for various exposure time (N=3)

N: growth medium prepared with environmental media (sea-water, soil, and $PM_{2.5}$ extracts)+ tested CBNs (incubation time 24 h).

N/No*100=100 means viability of bacteria were not affected by the exposure of CBNs, a lower (N/No*100) value reflects microbial toxicity of CBNs and a high (N/No*100) value shows nutrional effect on bacteria growth.

Results and discussion

To investigate the physicochemical transformations in different environmental media, the commercially purchased MWCNTs and GNPs were extensively analyzed with FTIR spectroscopy, SEM, dynamic light scattering and sedimentation.

FTIR spectroscopy is widely used to characterize the functional chemical groups on the surface of the CBNs, and this was mostly ignored in environmental evaluations of the nanomaterials. Also, it is known that co-ions of the media can be absorbed or adsorbed to the surface of the nanomaterials, or, or clean the impurities on the surface (Sperling and Parak 2010, Gawande et al. 2012, Faure et al. 2013, Baysal et al. 2018). According to FTIR spectrum in Figs 1 and 2, the most functional groups on the surface of CBNs are similar in the environmental media and in the control, however, the changes on the intensities and some new chemical formations were observed depending on the environmental media and their concentration. As can be seen in Fig 1, the main changes on the surface of MWCNTs were obtained by the soil media. The formation of S-O, C-N, C-S and -OH on the MWCNTs surfaces at 1100 cm^{-1}, 1200 cm^{-1}, 1300 cm^{-1} and 3500 cm^{-1} approved the surface modification by media. The media concentration also influence on the surface chemistry, for example, there was no N-related peak in the low concentration of soil as a result there was no N-related compounds in these media (Table 2). With the presence of N-related compounds at high concentration of soil media, the peaks appeared on the surface. Similar with the soil media, the presence of the peaks at 1300–3500 cm^{-1} and 3000–3500 cm^{-1} showed the effect of $PM_{2.5}$ and sea water on the surface of MWCNTs, respectively. The FTIR spectra also suggested that the GNPs changed in similar way after the environmental media treatment (Fig. 2), except for the -OH bands which decreased with the concentration of the soil and $PM_{2.5}$ while the -OH bands increased with the concentration of sea water.

Figs 3 and 4 give the surface morphology of the MWCNTs and GNPs using SEM, respectively. In soil, the MWCNTs are less entangled and their particle size decreased compared to the control. Between the soil concentrations, the MWCNTs were tighter in a high concentration of soil media. Contrary results were obtained for $PM_{2.5}$ and sea water, and the thinner nanotubes were shown in a high concentration of these media according to the SEM images, but the gaps increased in high concentrations. As shown in Fig. 4, the GNPs are plainer in the control and in a low concentration of soil media. However, bigger aggregates were obtained in a high concentration of soil media and cutting edge was shown. Similar results were

obtained for the $PM_{2.5}$ and sea water, and agglomerations were shown in a high concentration of $PM_{2.5}$ and sea water compared to their low concentrations. Especially dramatic differences were shown in a high concentration of sea water.

Also, the sedimentation behavior was investigated by UV-VIS (Fig 5). The MWCNTs and GNPs showed different sedimentation behavior. The MWCNTs pended on the test solution. As can be seen in Fig. 5, there was no or slight decrease in the sedimentation during the time interval. The low concentrations of soil and $PM_{2.5}$ behaved similar with the control. Besides that similar sedimentation behavior obtained in applied concentrations of sea water and also high concentration of $PM_{2.5}$. The sedimentation rate of the GNPs did not change in the low and high concentration of soil when compared to the control. However, the sedimentation rate decreased dramatically in the sea water and high concentration of $PM_{2.5}$ when compared to the control. The sedimentation was dominantly affected by low pH and electrolytes in the sea water (Zhao et al. 2014). As a result of the rapid aggregation of CBNs treated with sea water, surfaces could not be interacted

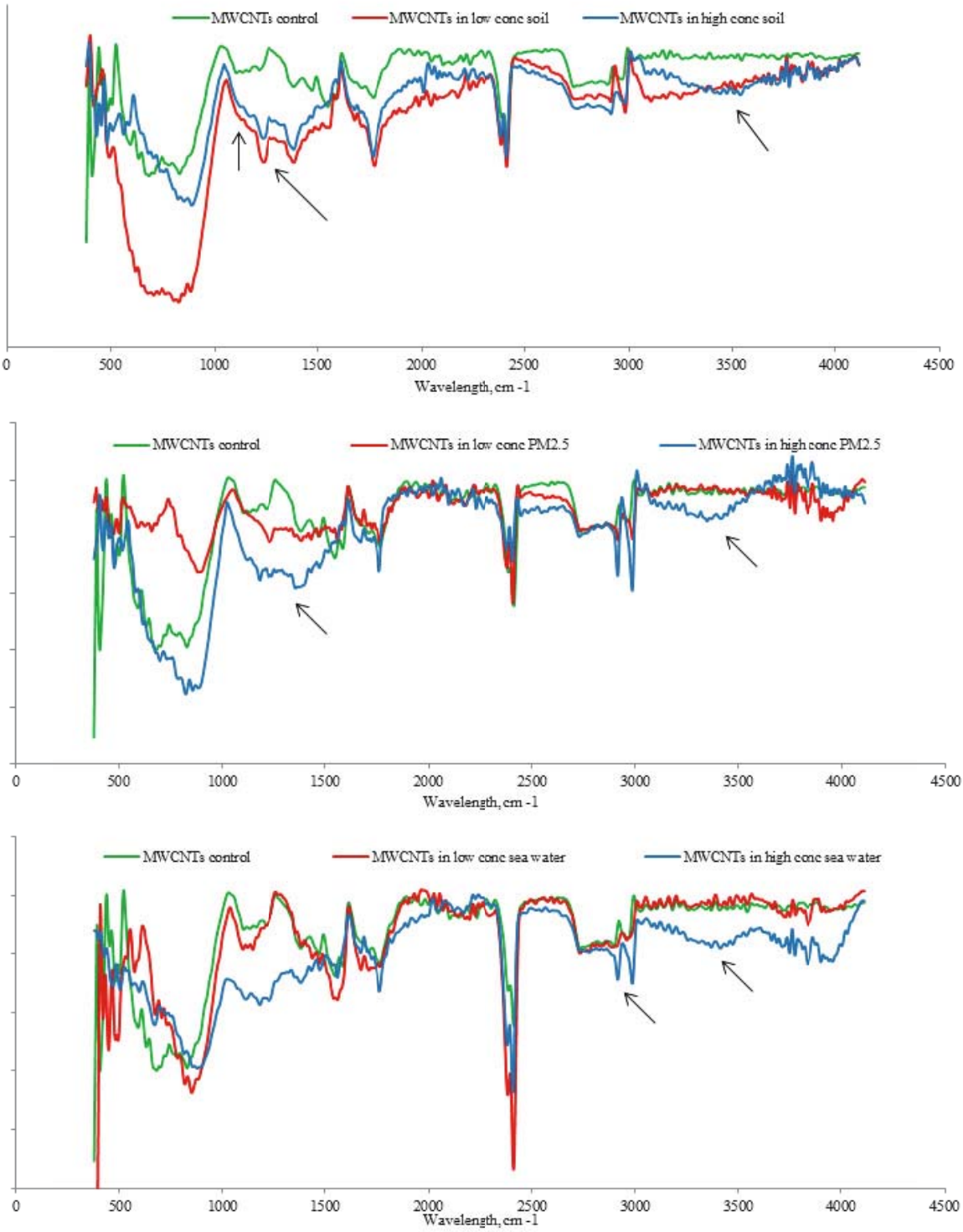


Fig. 1. FTIR spectrum of multiwalled carbon nanotubes dispersed in different environmental media (exposure time:24 h, N:5)

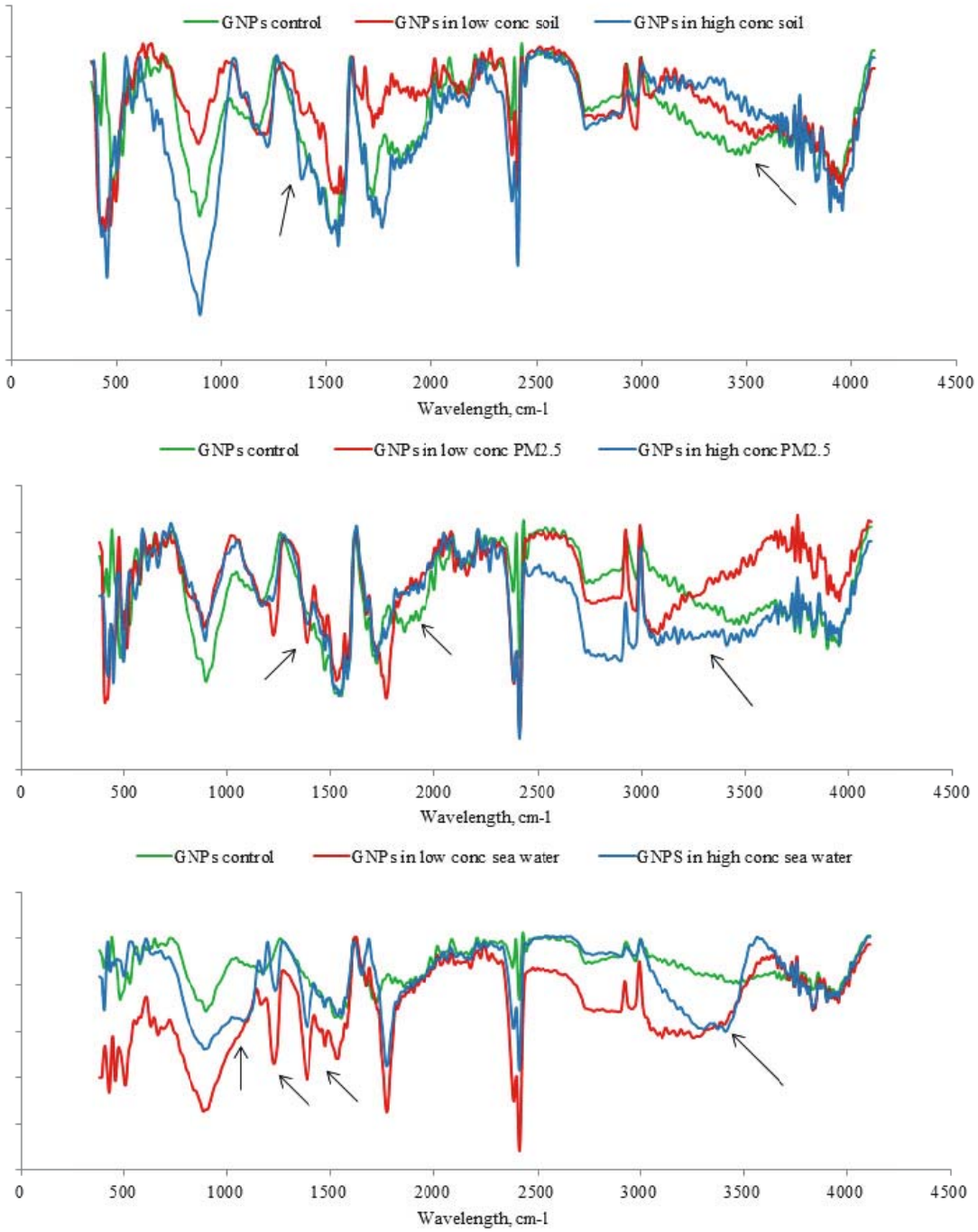


Fig. 2. FTIR spectrum of graphene nanoplatelets dispersed in different environmental media (exposure time:24 h, N:5)

with media chemical compounds, thus the adsorption of the new functional groups or the cleaning of the impurities on the surface can be lower by the exposure of sea water compared to the other environmental media.

Table 3 summarizes the particle size and zeta potentials of the CBNs treated with environmental media. The zeta potentials of the GNPs changed in the environmental media compared to in the controlled condition. While there were slight changes observed in both the low and high concentrations of the sea water, the high zeta potentials (above agglomeration level>10–15 mV) were obtained by the $PM_{2.5}$ and soil media. The zeta potentials increased at the lower concentration of $PM_{2.5}$ and soil, which means that the stability of the GNPs occurred in the low concentration of the $PM_{2.5}$ and soil media. Similar results were obtained for the MWCNTs and the zeta potentials decreased with the increasing concentration of the environmental media. As shown in the FTIR spectrums (Figs 1and 2), the main reason for the change could be the presence of the co-ions (sulfate, ammonia, carbonate etc.) in the media and/or media components/contaminants such as organic carbon, carbonate, amins and the hydroxyl group. etc. (Peng et al. 2017, Joo and Zhao 2017). Despite its importance in determining nanomaterial surface charge, the macromolecules or ligands (e.g. phosphate, nitrate and carbonate) in the media have been widely ignored in the studies on nanomaterial surface potentials and aggregation/agglomeration. However,

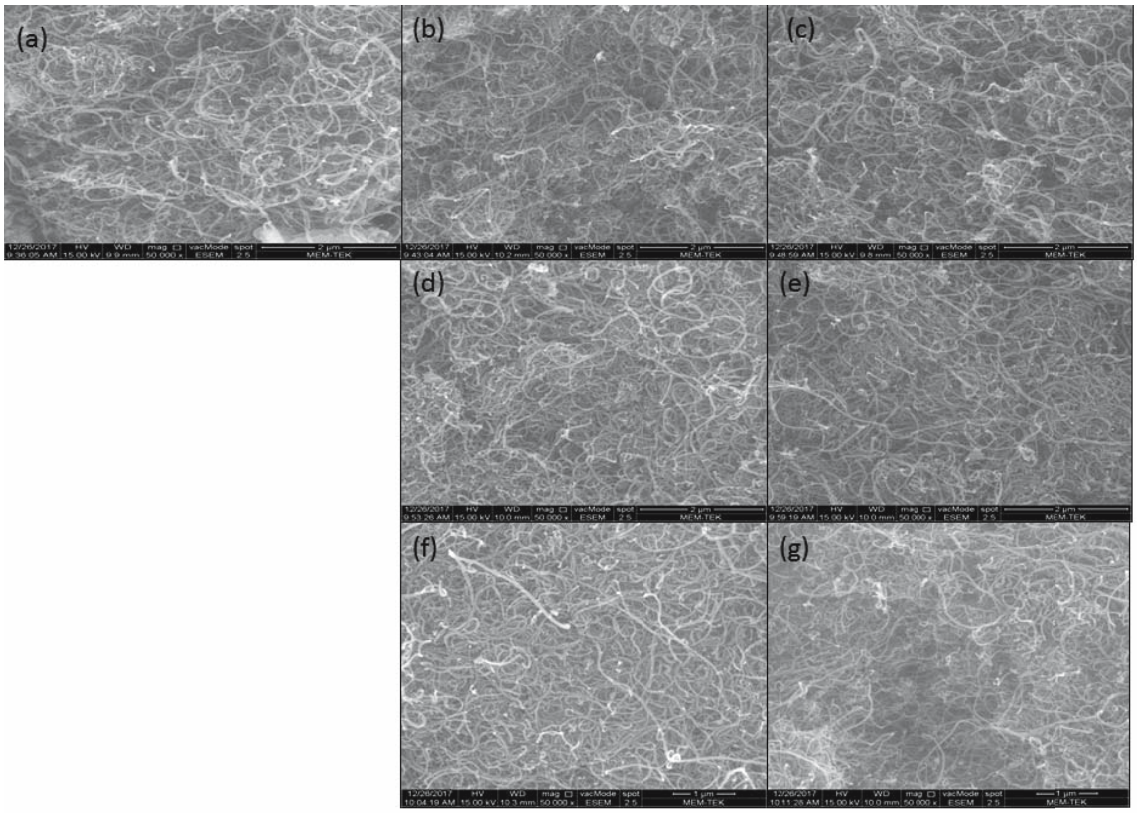


Fig. 3. SEM images of multiwalled carbon nanotubes; (a) MWCNTs in control, (b) MWCNTs in low concentration of soil, (c) MWCNTs in high concentration of soil, (d) MWCNTs in low concentration of PM2.5, (e) MWCNTs in high concentration of PM2.5, (f) MWCNTs in low concentration of sea water, (g) MWCNTs in high concentration of sea water

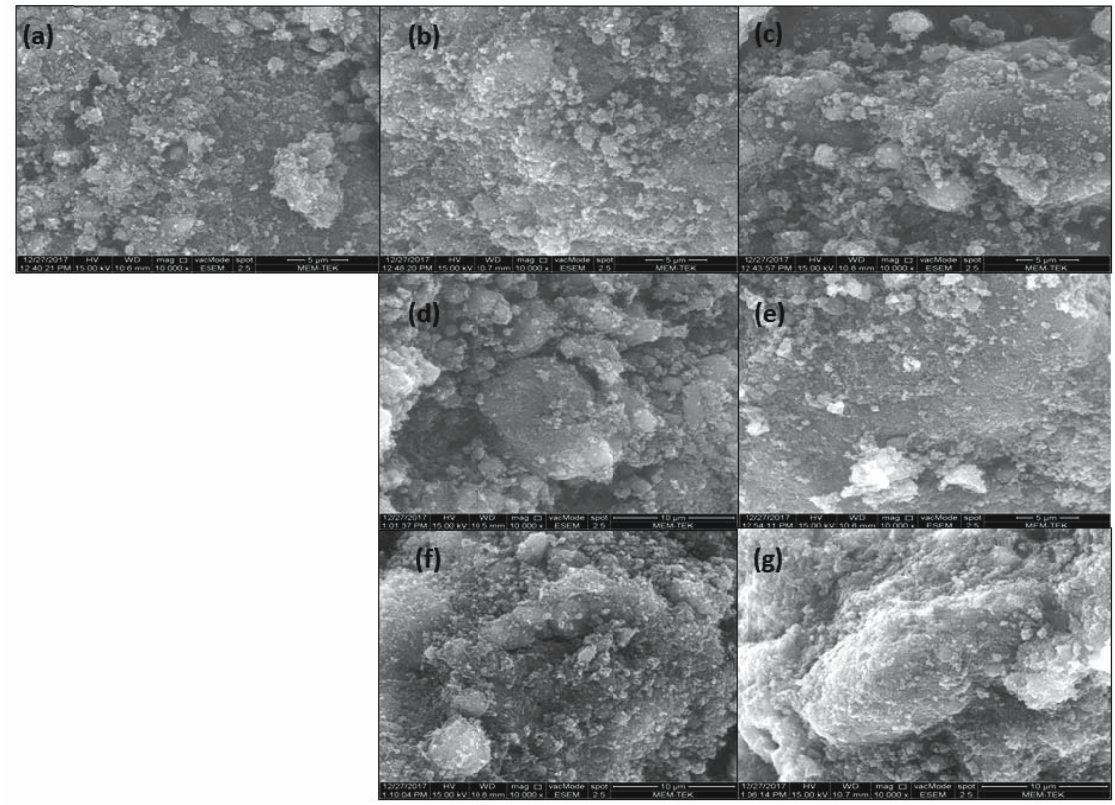


Fig. 4. SEM images of graphene nanoplatelets; (a) GNPs in control, (b) GNPs in low concentration of soil, (c) GNPs in high concentration of soil, (d) GNPs in low concentration of PM2.5, (e) GNPs in high concentration of PM2.5, (f) GNPs in low concentration of sea water, (g) GNPs in high concentration of sea water

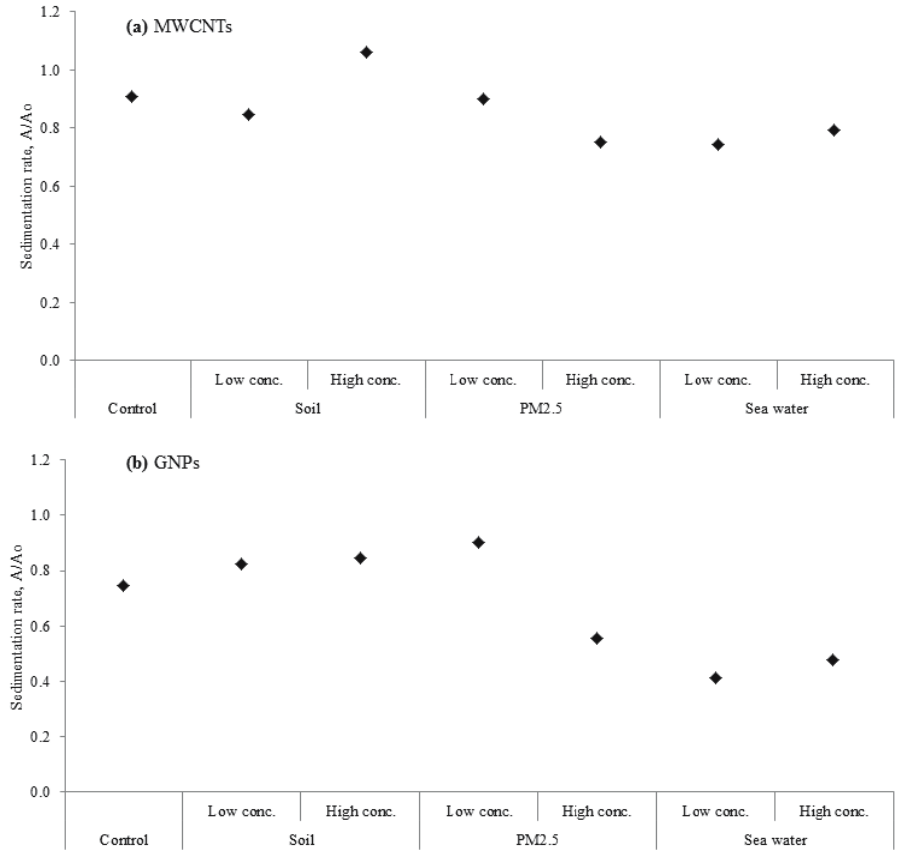


Fig. 5. Sedimentation rate of (a) MWCNTs, and (b) GNPs in control and in different environmental media (Sedimentation rate=A/Ao, sedimentation time: 0 for Ao, and sedimentation time:24 h for A, N:5)

Table 3. Comparison of the hydrodynamic size and zeta potentials of GNPs and MWCNTs in control condition and in different environmental media (exposure time:24 h, N:3)

Nanomaterial	Parameters	Control	Soil		$PM_{2.5}$		Sea water	
			Low conc.	High conc.	Low conc.	High conc.	Low conc.	High conc.
MWCNTs	Zeta potentials, mV	12.1±0.8	23.7±0.9	21.8±0.7	22.9±2.1	18.5±1.4	13.6±0.7	8.0±0.5
	Hydrodynamic size, nm	110±9	206±18	80±7	489±23	85±6	108±7	200±14
GNPs	Zeta potentials, mV	13.4±0.4	25.3±1.1	22.1±0.9	32.2±1.2	20.3±1.7	13.4±0.8	12.0±0.3
	Hydrodynamic size, nm	632±14	193±17	62±5	102±11	162±13	74±7	715±34

the latest studies report the importance of chemical interactions in addition to physical interactions in the nanomaterial surface potential and aggregation/agglomeration (Baalouska 2017, Metreveli et al. 2016). In the study performed by Afshinnia et al. an increased concentration of co-ions such as carbonate and phosphate anions in the medium were decreased in the zeta potentials of Ag NPs (Afshinnia et al. 2017). In our study, the high zeta potentials of the MWCNTs and GNPs were obtained in the $PM_{2.5}$ and soil media compared to the control due to the positively charged functional groups (N-H, C-N etc.) induced with these media. In addition, the zeta potentials decreased with the increased concentration of the environmental media resulting from the increasing negatively charged functional groups (S-H, -OH etc.). Nevertheless, the changes of zeta potential in the sea water were found to be smaller than in other media because the adsorption of the new functional groups or the cleaning of the impurities on the surface were lower by the exposure of sea water due to the high sedimentation rate compared to the other environmental media.

The hydrodynamic size of the CBNs had a slight negative correlation with the zeta potentials, and the high zeta potentials increased the stability and a lower size was obtained. The comparison between the controlled conditions and the different environmental media results shows that the size of the GNPs decreased in the low concentration of $PM_{2.5}$ and sea water with the high zeta potentials. The exposure of the low concentration of sea water and $PM_{2.5}$ media supported the internalization of the GNPs. Also the results indicated that components of the sea water were more effective on the particle size of GNPs compared to the other media. On the other hand, the size of the MWCNTs increased with the high concentration of sea water as a result of decreasing the zeta potential. Contrary results

were observed in the $PM_{2.5}$ and soil media, and significant size decreases were measured through the increasing concentration of the $PM_{2.5}$ and soil, whereas the zeta potentials decreased. Moreover, although particles sizes were decreased, the gaps were obtained according to the SEM images.

The toxicity of the GNPs and MWCNTs in different environmental media (soil, $PM_{2.5}$ and sea water) was investigated regarding the bacteria. Despite there being no bacteria inhibition at 24 h as an exposure time of the GNPs and MWCNTs in the controlled conditions, the viability was affected by the environmental media type and its concentration. As can be seen in Fig 6, *E. coli* and *S. aureus* did not show any inhibition to the GNPs and MWCNTs dispersed in the soil media, unless the *P. aeruginosa* was inhibited by the high concentration of soil media including 25 mg/L MWCNTs (80% inhibition). Also, *B. Subtilis* was strongly affected by the high concentration of the soil media including the MWCNTs. Similarly with the MWCNTs, *E. coli* was more resistant to the GNPs in the soil media. The GNPs showed an approximate 10–20% decrease in viability in the *P. aeruginosa* and *S. aureus* in a high concentration of soil including 25 mg/L GNPs, and *B. subtilis* was more susceptible in low and high concentration of soil including 25 mg/L GNPs; the 20% and 60% inhibitions were observed in low concentrations and high concentrations of soil including 25 mg/L GNPs, respectively. The viability results show that *E.coli* as gram--negative bacteria and *S. aureus* as a gram-positive bacteria were more resistant to the CBNs dispersed in the soil media.

The behavior of the CBNs in the $PM_{2.5}$ is shown in Fig 7. The viability of *S. aureus* and *B. subtilis* dramatically decreased with the exposure to all the tested concentrations of the MWCNTs (2.5–25 mg/L MWCNTs) being dispersed into the high concentration of $PM_{2.5}$. The 80–100% inhibition occurred in these media. *E. coli* and *P. aeruginosa* as gram-negative bacteria did not show any inhibition. In addition, the GNPs showed an approximate 20% and 40% decrease in viability in the *B. subtilis* against 25 mg/L GNPs in the low and high concentrations of $PM_{2.5}$, respectively. The same results for the viability were obtained compared to the soil media, *E.coli* and *P. aeruginosa* as gram-negative bacteria were more resistant to the $PM_{2.5}$ media including GNPs. On the other hand, there was no inhibition in *S. aureus* in the $PM_{2.5}$ media including the GNPs when compared to the MWCNTs. Also *B. subtilis* had inhibition to the CBNs dispersed in $PM_{2.5}$.

The effect of sea water on the behavior of CBNs is depicted in Fig 8. The viability behavior of the MWCNTs in the sea water was more effective in the microorganism when compared to the other media. While exposure to the MWCNTs in a low concentration of sea water did not exhibit any inhibition in the bacteria, the *E. coli, P. aeruginosa, S. aureus and B. subtilis* viability decreased in the MWCNTs in the high concentration of sea water (40–80% inhibition). The bacteria strains were more resistant to the GNPs that were dispersed into the sea water. The inhibition was observed only in the *P. aeruginosa* as gram-negative bacteria (5–25 mg/L). A 50% inhibition of *P. aeruginosa* occurred in the high concentration of sea water according to the results.

Our study revealed that *i*) MWCNTs are more toxic to the tested bacteria than GNPs, *ii*) Gram-negative *E. coli* and gram--positive *B. subtilis* are more stable than *P. aeruginosa* and *S. aureus, iii*) MWCNTs and GNPs in $PM_{2.5}$ had more inhibition effect on *S. aureus, P. aeruginosa* and *B. subtilis*. MWCNTs and GNPs in soil were more vulnerable to *B. subtilis* and *P. aeruginosa* than other tested bacteria, *iv*) the MWCNTs dispersed in the sea water were more effective on the inhibition

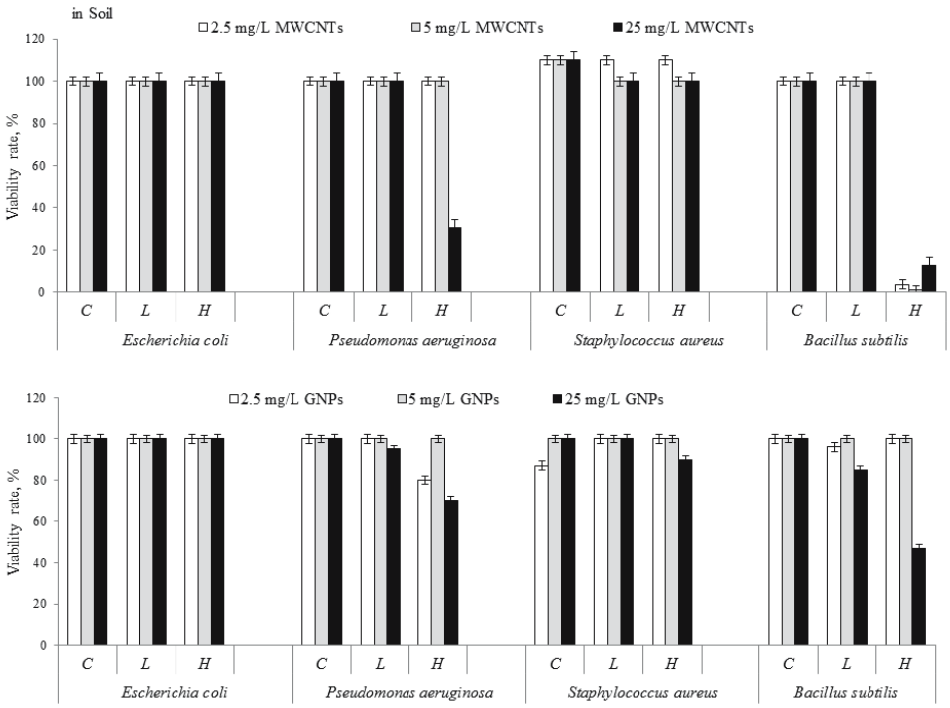


Fig. 6. Growth viability rates of microorganism exposed to low and high concentration of soil with various concentration of GNPs and MWCNTs (C:control, L: low concentration, H: high concentration of soil; exposure time:24 h, N:5). Values expressed as mean±standard deviation

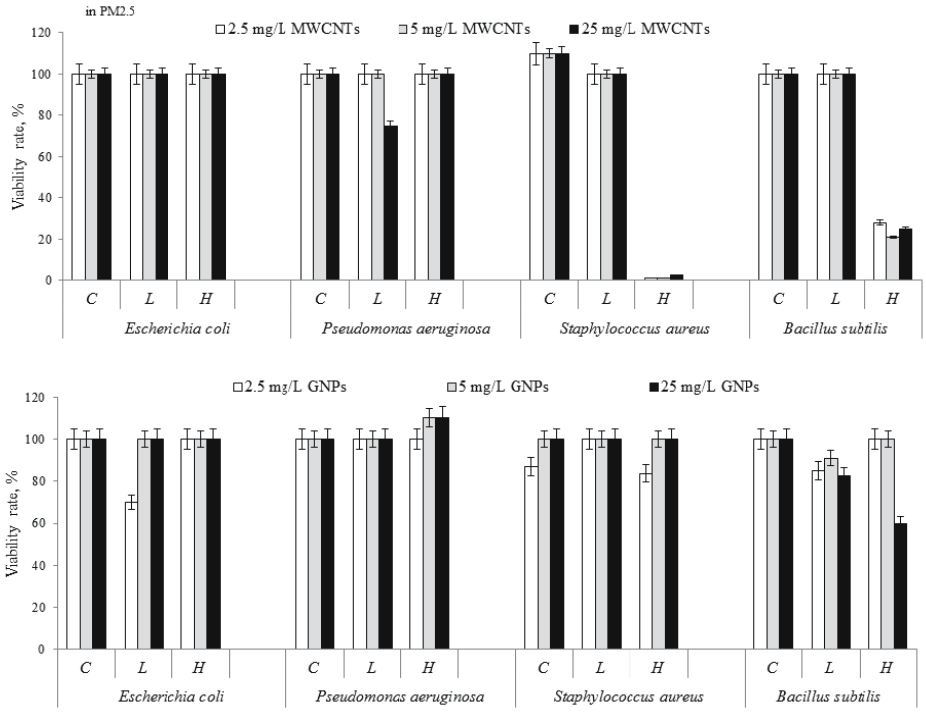


Fig. 7. Growth viability rates of microorganism exposed to low and high concentration of PM2.5 airborne particulate with various concentration of GNPs and MWCNTs (C: control, L: low concentration, H: high concentration of PM2.5 airborne particulate; exposure time: 24 h, N:5). Values expressed as mean±standard deviation

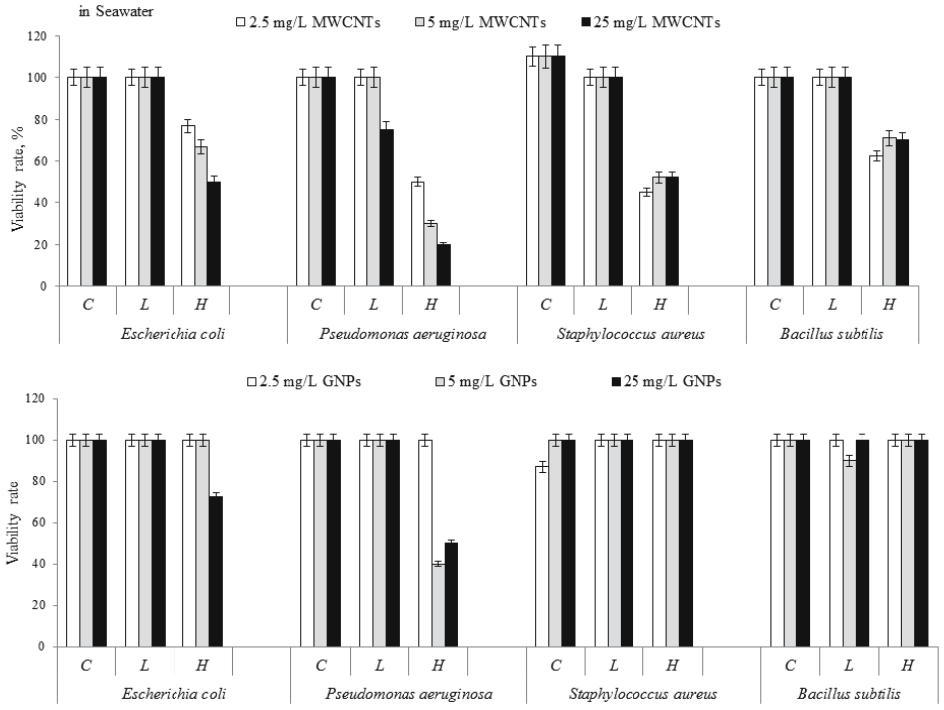


Fig. 8. Growth viability rates of microorganism exposed to low and high concentration of sea water with various concentration of GNPs and MWCNTs (C: control, L: low concentration, H: high concentration of sea water; exposure time: 24 h, N:5). Values expressed as mean±standard deviation

degree of the bacteria, as well as the inhibited bacteria diversity compared to the other media, v) the high concentration of the environmental media was found to have a more inhibitory effect on the bacteria viability.

The viability results indicated that the MWCNTs were more effective in the bacterial inhibition compared to the GNPs. While the MWCNT was the closest material to the GNPs, the graphene and its derivatives contained many oxygen

atoms in the forms of carboxyl groups, epoxy groups, and hydroxyl groups. The toxicity was related to the abundance of the oxygen atoms, and the abundant oxygen decreased in its toxicity (Zhao et al. 2014, Akhavan and Ghaderi 2010).

Moreover, if tested CBNs were inhibited to the selected bacteria in controlled condition, the first and conventional explanation for the inhibition could be the nature of the bacteria cell walls and charge differences between bacteria cell and CBNs. However, in our study, there was no inhibition in controlled condition, and the decrease on the viability found with the environmental media. Thus, in our cases, the selected gram-negative bacteria showed inhibition from the exposure of the CBNs in the environmental media as a consequence of the increased difference in the surface charge between the CBNs and bacteria. Electrostatic repulsion between positively charged CBNs and the positively charged surface of bacteria (*B. Subtilis, S. aureus* etc.) may avoid association between bacteria and CBNs, and consequently limit their toxicity. Unless the zeta potentials had the same charge, the inhibition occurred with the exposure to the environmental media. The main reason for the bacterial inhibition could be the changing of physicochemical properties such as surface chemistry, sedimentation, zeta potential and/or particle size through exposure to the media. Under these circumstances, the surface chemistry is the main factor affecting other tested physicochemical properties, as well as on the inhibition of bacterium.

While the zeta potentials and particle size play an important role in the toxicity of the nanomaterials as they largely define their interactions with the biological systems, their behavior can be changed in different suspensions. Especially small size of nanomaterials and reactivity can cause penetration into the tissues and interfere bacterial biochemical cycles (Zapor 2016, Krzyzewska et al. 2016). In particular, the chemical compounds in the media affect the zeta potential, particle size and sedimentation. It was a major determinant in the colloidal behavior; it specifically influences the organism response upon exposure to the nanomaterial by changing its zeta potential, shape and size through an aggregate or agglomerate formation. Furthermore, the sedimentation of CBNs dispersed in the sea water seemed to be more influential on the inhibition of bacteria. As a results of electrolytes and pH which decreased the surface charge of CBNs incresed the sedimentation (Zhao et al. 2014).

Conclusion

The CBNs exhibited distinct physicochemical properties in different environmental media which consequently led to variation of bacterial toxicity. The changes of physicochemical properties dominated through chemical compounds of each environment. Environmental media affect the physicochemical properties of the CBNs and the inhibition degree of the bacteria especially by the changing of the intensities and the formation of functional groups on the surface.

Also the results showed that bacterial distribution can change with the interaction of CBNs with environmental media. Thus, it is important to obtain the bacterial balance for the environment, as well as for human health. From this result, toxicity should be taken into specific consideration using chemical components of environmental media. Therefore, the generalization of the toxicity of the CBNs on the environment must be avoided using controlled conditions, exposure duration, etc. Since the concentration of chemical constituent of environmental media influences the nanomaterial behavior, environmental media characteristics needs extra investigation and to suggest the allowable limit for the CBNs, the regional chemical composition can be taken into account.

We believe that the data presented in this paper would have the potentiality to be used in the field of environmental risk assessment and, as a result, benefit human health. This study also suggests the potential of using alternative and sensitive toxicity bacteria model system towards *P. aeruginosa* as gram negative bacteria and *B. subtilis* as gram positive bacteria can be used in screening the toxicity of such nanomaterials.

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