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Ecotoxicity evaluation of Cu- and Fe-CNT complexes based on the activity of bacterial bioluminescence and seed germination

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ABSTRACT

The toxic effects of the composites of Fe^0 and Cu^0 with different percentages of CNTs were examined based on the activity of bacterial bioluminescence and seed germination. In terms of the EC_{50} values, the toxic effects of Cu^0 on bacterial bioluminescence and seed germination were approximately 2 and 180 times greater than that of Fe^0, respectively. The toxicity increased with increasing CNT content in the Cu-CNT mixtures for both organisms, whereas opposite results were observed with Fe-CNT mixtures. The mean toxic effects of Cu-CNT (6%) were approximately 1.3–1.4 times greater than that of Cu-CNT (0%), whereas the toxic effects of Fe-CNT (6%) were approximately 2.1–2.5 times lower than that of Fe-CNT (0%) for both the bioluminescence activity and seed germination. The causes of this phenomenon are unclear at this point. More research will be needed to elucidate the mechanism of the toxicity of nano-mixture materials and the causes of the different patterns of toxicity with Cu- and Fe-CNT mixtures.

Introduction

Nanotechnology has emerged as an enabling technology with high potential impact on virtually all fields of mankind. Estimates suggested that by 2015 nanotechnology will have a trillion-plus dollar global economic impact (Ge et al., 2012). Berman (2016) also reported that nanotechnology will bring improvements by addressing environmental problems using advanced nanotechnology by 2025. The implementation of unique materials and devices ranging from electronics to engineered tissues is one of the rapidly growing fields of nanotechnology (Shvedova et al., 2003). Two advanced nanomaterials that are being used increasingly for different technologies are carbon nanotubes (CNTs) and graphene. CNTs are well-ordered, high aspect ratio allotropes of carbon. CNTs have attracted considerable interest from both scientists and industry because their unique atomic configuration, mechanical, optical, electronic properties, high aspect ratios (*e.g.*, like fibers), strength, and remarkable physical properties (Smart et al., 2006). Current global multi-wall CNTs production capacity is estimated to be 13,996 tons (Zhao et al., 2017). Carbon nanotube metal matrix composites are an emerging class of new materials that are being developed because of the high tensile strength and electrical conductivity of carbon nanotube materials.

Powder metallurgy processing to prepare nanocrystalline CNT composite powders with Cu and Fe have attracted considerable attention in the next generation automobile industry to manufacture the desired advanced automotive

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components because of their promising mechanical, electrical, and thermal properties. Any exposure of humans to CNTs would pose considerable danger to human health. Therefore, understanding and ensuring the safety of nanomaterials are of huge importance to the tremendous commercial applications of nanotechnology (Foldvari and Bagonluri, 2008; Tang et al., 2012). Safety evaluation of nanomaterials should consider their behaviors in various aspects, including their interactions with proteins, DNA, lipids, membranes, organelles, cells, tissues, biological fluids, and even the dissolution of metal constituents (Zhao et al., 2008; Nel et al., 2009; Liu et al., 2013). There are different ways, in which the human body can be exposed to nanomaterials, including penetration through the skin, ingestion, inhalation, and injection (Zhao and Liu, 2012).

A great effort in recent years has been performed to elucidate the mechanism of nanotoxicity in living organisms and cells. For this purpose, scientists and researchers have examined the toxicity of CNTs in several cell lines (Wang et al., 2011; Pichardo et al., 2012). Their efforts suggest that various factors, including functioning, dimensions, and characteristics, can determine the toxicity of CNTs, including environmental factors, such as temperature, humidity, and barometric pressure, affecting rates of consumption and even the occurrence of some toxic agents (Horie et al., 2012). Recently, *in vivo* toxicology studies, by focusing on multiple organ systems, have proved that CNTs can cause a toxic response within these multiple organ systems. Research into the ecotoxicity of nanomaterials has demonstrated different levels of toxicity depending on the bioassays (Brunner et al., 2006; Soto et al., 2006). TiO_2 and ZnO nanomaterials, which are used widely in sun protection products as well as self-cleaning coatings, have been shown to have toxic effects that can inhibit the growth of microalgae, crustaceans, and bacteria, while opposite results have been reported by other investigators (Serpone et al., 2007; Heinlaan et al., 2008; Aruoja et al., 2009). Ecotoxic bioassays using various test organisms (bacteria, algae, protozoa, plants, and fish) and their metabolic processes have gained widespread attention for environmental contaminants (Banks and Schultz, 2005). Knowledge of each test organism's sensitivity is important for evaluating the contaminant toxicity levels. Among these processes, seed germination and bacterial bioluminescence were adopted because of their high sensitivity, simplicity, *etc.* Plant toxicity assays are particularly relevant when the phytotoxic contaminants of nanomaterials are present in soil (Boutin et al., 2004). Among plant processes, seed germination studies are considered short-term because they assess the rapid response to acute toxicity. The root and shoot elongation test is one of the simplest short-term methods used in environmental biomonitoring (Di Salvatore et al., 2008). Assays based on bacterial bioluminescence are a time-saving and cost-effective test that are used widely as a reproducible and sensitive screening method for determining the acute toxicity of different sample types (Wang et al., 2002).

In this study, composites of Fe^0 and Cu^0 with different percentages of CNTs, which are currently used in laboratories and the automobile industry without any restriction by environmental law, were tested. The ecotoxic effects of composites of Fe and Cu with CNTs were evaluated based on the activity of bacterial bioluminescence and seed germination.

1. Materials and methods

1.1. Preparation of metals-CNT mixtures

CNTs were synthesized using a catalytic CVD method and then dispersed in distilled water at concentration of 5 wt.% CNT using a Sonosmasher. Carboxymethyl cellulose (CMC) was used as the dispersant with a 9 sec working interval and 1 sec off continuously, totaling 6 hr in dispersion for the finally dispersed product. Copper powder with a particle size of up to 63 μm was purchased from Markin Metal Powders Ltd. (UK). Iron powders with a particle size of up to 150 μm were purchased from Hoganas Ltd. Each metal powder was placed into a milling chamber with the CNTs solution for the attrition ball milling process using SUS-316 L balls, 5 mm in diameter, as the milling media. Ball milling was proceeded at 300 r/min for 1 hr. The milled solution was then dried in an oven for 24 hr to produce the final milled and mixed metal-CNTs powder. Four different conditions of the mixture samples were prepared according to the CNT concentrations: 0, 1%, 3%, and 6% (W/W).

1.2. Toxicity test of metals-CNT mixtures on bioluminescence activity

The toxicity of the metal-CNT mixtures was measured based on a bioluminescence activity of the *Escherichia coli* DH5α strain RB1436, harboring a variant of the pUCD615 plasmid (obtained from R. Burlage, Concordia University, USA). This strain, which contains a constitutive promoter to express the *lux* genes, produces bioluminescence in a growing culture and is used to detect deleterious conditions that would cause a measureable decrease in bioluminescent output, such as those induced by metal-CNT mixtures. The RB 1436 strain was stored at −70°C until needed, at which time, it was grown overnight in Luria–Bertani^{ka} (LB^{ka}) medium at 27°C with shaking at 130 r/min. The culture was diluted 30-fold in LB^{ka} medium and allowed to grow until the optical density (OD_{600}) reached approximately 0.6. This culture was diluted appropriately with minimum salt medium to a final OD_{600} of 0.2 for the toxicity test (Ko and Kong, 2014). For the test, 1 mL of the diluted bacterial suspension was mixed with 9 mL of the sample, after which the bioluminescence activity was measured after 1 and 1.5 hr of incubation. The bioluminescence activity was measured using a Turner 20/20 luminometer (Turner Design, USA), which had a maximum detection limit of 9999 relative light units (RLU).

1.3. Toxicity test of metals-CNT mixture on seed germination

The seed (*Lactuca sativa* L.) produced and distributed by a commercial seed company (Nongwoo Bio., South Korea) were purchased from a local seed store. These particular seeds were employed in the test because the plants from which they were obtained are important food crops in the local region. Prior to the germination test, all seeds were surface-sterilized with an aqueous 3% H_2O_2 solution for 10 min and then rinsed with distilled water. Filter paper was then placed in a sterilized Petri dish and moistened with 5 mL of an aqueous solution (distilled water for control) containing the metal-CNT mixtures. Twenty seeds from each species were placed in each plate, which was then covered with a lid and incubated in the dark at (23 ± 2)°C. The germinated seeds were then counted after 3 days of incubation. An extension of both the plumule and radicle to longer than 2 cm from their junctions was considered to be indicative of germination. Three replicates were performed for each treatment.

1.4. Statistical analysis and soluble metal determination

The EC_{50} (concentration of chemical at which 50% of its effect is observed) values of the Cu and Fe particles were normalized to the control activity using the Trimmed Spearman–Karber method. The EC_{50} values were estimated using the SPEARMAN computer program, which is distributed by the US-EPA's Center for Exposure Assessment Modeling (CEAM). The Student's *t*-test (http://www.graphpad.com) was used for statistical analysis of the experimental groups. Each of the experimental values was compared with its corresponding control. Statistical significance was accepted when the probability of the result assuming the null hypothesis (p) was less than 0.05.

The metal-CNT mixtures were suspended directly in deionized water (pH 7.8) and dispersed by ultrasonic vibration for 10 min prior to use. Metal ions in the metal-mixture solution were determined at the end of the incubation period of the test for the bacterial bioluminescence activity. The solution samples were filtered (0.45 μm, advantec MFS Inc., CA, USA) after incubation to determine the solubilized metal ion concentration in the solutions. An inductively coupled plasma optical emission spectrometer (Optima 7300DV ICP-OES, Perkin-Elmer Inc., USA) was used to determine copper and iron concentrations in the sample solution. The instrumental operating conditions and the interference free emission lines of each element are as follows: RF generator power (1.65 kW), plasma gas flow rate (15 L/min), auxiliary gas flow rate (0.2 L/min), nebulizer gas flow rate (0.65 L/min), solution uptake rate (1.5 L/min), and analytical lines (Cu 324.754, Fe 238.204 nm).

2. Results and discussion

2.1. Synthesis and characteristics of metal-CNT complexes

The dispersion of CNTs in solution is important. Because CNTs are hydrophobic, the introduction of hydrophilic groups, such as hydroxyl or carboxyl groups, on the surface of the CNTs and/or the addition of dispersants of CNTs is necessary to disperse the CNTs in aqueous solutions, as shown in Fig. 1. The following ultra-sonic mixing of copper or iron powder and CNTs in an aqueous medium provides an opportunity to generate homogenous well-dispersed powder mixtures, as shown in Fig. 2. The mixture powders were prepared for an investigation for the toxicity test. In addition, these mixtures could be consolidated by hot pressing, spark plasma sintering and hot isostatic pressing to produce a solid. To manufacture-desired advanced automotive components, powder metallurgy processing with the prepared nanocrystalline CNT, and graphene composite powders with Fe and Cu have attracted considerable attention for the next generation automobile industry owing to their promising mechanical, electrical and thermal properties (Smart et al., 2006). Copper is a ductile metal with very high thermal and electrical conductivity. In sufficient concentrations, Cu compounds are toxic to higher organisms and are used as bacteriostatic substances, fungicides, and wood preservatives. The CNTs dispersed in the Cu-matrix composite impart significantly enhanced physical and mechanical properties, such as friction, hardness, toughness, and wear resistance. A certain proportion of carbon (0.002%–2.1%) in a Fe-matrix produces steel, which may be up to 1000 times harder than pure iron. Fe-CNT composites can disturb the metastable equilibrium in the nanoscale Fe–C system between carbide and graphite phases during iron crystallization inside graphite tubular channels (Zhao et al., 2008).

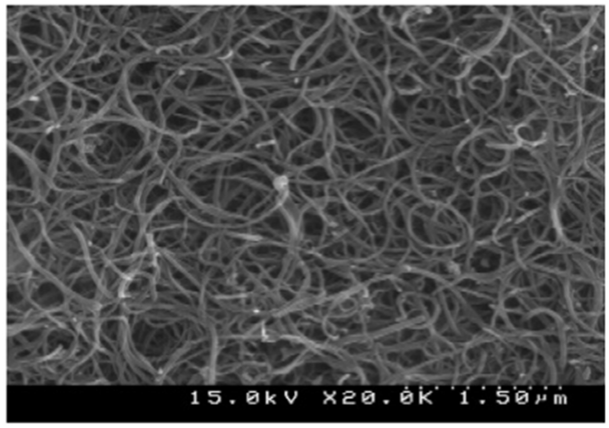

Fig. 1 – Dispersed CNTS in aqueous solutions before mixing with metal particles. CNTs: carbon nanotubes.

2.2. Effects of Cu^0 and Fe^0 particles on the activity of bioluminescence and seed germination

Prior to assessing metal-CNT mixture effects, the relative toxic effects of Cu and Fe particles without CNTs on the bioluminescence activity and seed germination were examined (Fig. 3). The dose ranges for each method and particle were set based on several preliminary tests. In the case of bacterial bioluminescence, the control (no exposure to metals) produced a mean bioluminescence in the range of (292 ± 58.2)–(570 ± 51.5) RLU after 1 and 1.5 hr of incubation depending on the condition of each set. Different bioluminescence activities were observed according to the type of metal mixtures tested. As shown in Fig. 3, the toxic effects of Cu particles on the bioluminescence activity were greater than those of Fe particles. For example, at 180 mg/L Cu and Fe particles (the lowest dose), the bioluminescence activity was 90% and 101% of the control, respectively. In contrast, at 900 mg/L Cu and Fe particles, the corresponding activity was 55% and 39% of the control bioluminescence activity (corresponds to 45% and 61% toxicity), respectively. The mean bioluminescence intensities after 1 and 1.5 hr of exposure were used to calculate the EC_{50} value for each metal. The EC_{50} values (95% confidence level range), which were calculated using the SPEARMAN program, were 723 (447.6–1168.3) mg/L and 1063 (875.0–1291.7) mg/L for Cu and Fe particles,

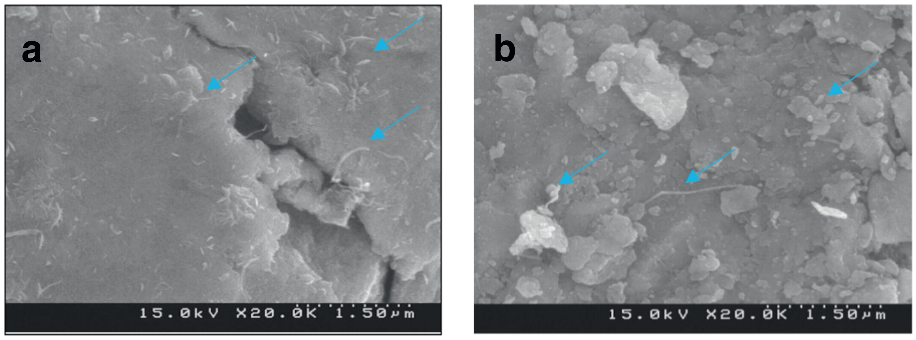

Fig. 2 – Homogenously dispersed CNTs/Cu powder mixture (a) and Fe powder mixture (b).

respectively (Table 1). Therefore, Cu particles showed approximately 1.5 times higher toxicity than that of Fe particles on the bacterial bioluminescence.

The relative toxicity of Cu and Fe particles without CNTs on seed germination were also examined (Fig. 3). The dose ranges for each metal for seed germination were set to 0–25 mg/L Cu and 0–2000 mg/L Fe based on several preliminary tests. Very different patterns of effect were also observed depending on the type and concentration of metals. For the control, forty to fifty (average 16 per batch of 20 seeds) out of 60 seeds germinated (longer than 2 cm growth) during a 3-day incubation period. As shown in Fig. 3, in the presence of Fe particles, almost no inhibition was observed up to 1000 mg/L Fe, and 44% seed germination of the control (56% toxicity) was observed at highest concentration tested (2000 mg/L). In the presence of Cu particles, however, almost complete inhibition was observed at 25 mg/L Cu (90% toxicity on germination). The EC_{50} values (95% confidence level range), which were calculated using the SPERMAN program, were 11 (9.3–12.9) mg/L and 1834 (1646.5–2044.3) mg/L for Cu and Fe particles, respectively. In terms of the EC_{50} values, the toxic effects of Cu were approximately 180 times greater than those of Fe on seed germination. The effects of these highly insoluble chemicals may be caused by the particle itself and solubilized metal ions *etc.* Heinlaan et al. (2008) reported that the solubilized metal ions from the metal oxide NPs may be responsible for the observed antibacterial effects because bacteria are largely protected against NP entry (no transport mechanisms for supramolecular and colloidal particles). The effects of chemicals on seed germination are dependent on their ability to reach embryonic tissues across physiological barriers, which are directly dependent on the seed coat structure and changes according to the physical and chemical properties of the pollutants. The inhibition of specific enzymatic reactions (*e.g.*, amylase) by chemicals also could be explain the chemical toxicity of seed germination (Seregin and Kozhevnikova, 2005; Akinci and Akinci, 2010).

2.3. Effects of Cu-CNT mixtures on the activity of bioluminescence and seed germination

The effects of Cu-CNT mixtures with different percentages of CNTs were evaluated based on the bacterial bioluminescence and seed germination (Fig. 4). Based on the EC_{50} values for Cu particles, the effects of the Cu-CNT complexes with different percentages of CNT were examined at 720 mg/L Cu-CNT for bacterial bioluminescence and 10 mg/L Cu-CNT for seed germination. In the presence of 0, 1%, 3%, and 6% CNTs, the relative toxicity was (58 ± 4.1)%, (62 ± 6.6)%, (70 ± 3.7)%, and

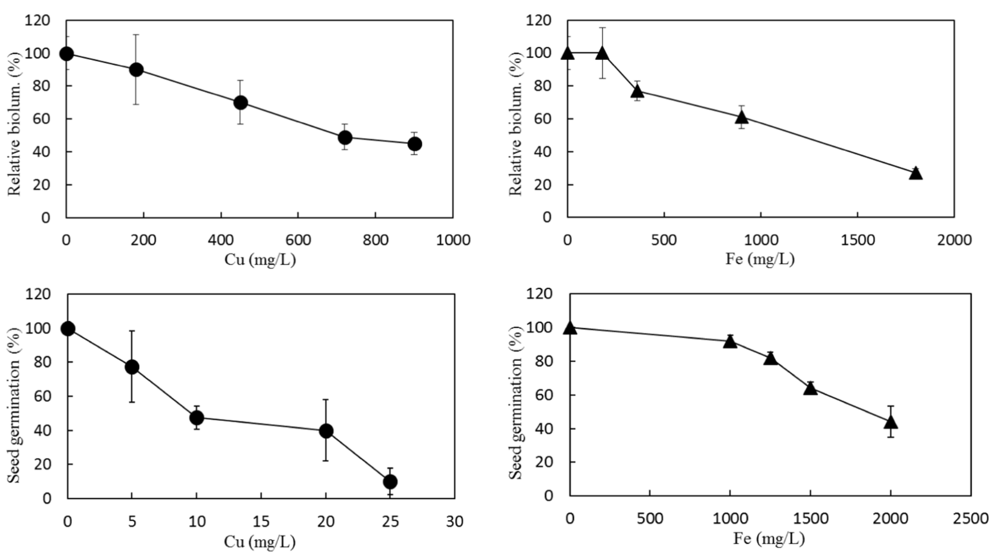

Fig. 3 – Effects of Cu^0 and Fe^0-particles on the bacterial bioluminescence and seed germination.

Table 1 – EC_{50} of metal particles based on the activity of bacterial bioluminescence and seed germination.

Metal particle	EC_{50} (mg/L)	
	Bioluminescence	Seed germination
Cu^0	723 (447–1168) *	11 (9.3–12.9)
Fe^0	1063 (875–1292)	1834 (1646–2044)

* Values presents ranges of 95% confidence levels.

(83 ± 16.9)% (avg. (68 ± 10.9)%) for bacterial bioluminescence and (41 ± 14.2)%, (45 ± 6.81)%, (45 ± 11.8)%, and (55 ± 7.87)% (avg. (47 ± 5.7)%) for seed germination, respectively. Therefore, the mean toxicity increased with increasing CNT content in the Cu-CNT mixtures for both organisms. Under conditions of 6% CNT, the toxic effects were approximately 1.3–1.4 times greater than that of 0% CNT for both the bioluminescence activity and seed germination. Causes of these phenomena are unclear at this point. On the other hand, the metals have different carbon solubility in the liquid state with their own standard atomic structures and crystal lattices with the CNTs. Those cases had proven exhibition of CNTs toxicology in the state of mixtures. Smart et al. (2006) reported that a large portion of the discrepancies in toxicity and biocompatibility data are due to differences in CNT dispersions, which is the factor that ultimately dictates the presentation of CNT to cells. By conventional criteria, however, the differences were not considered to be not quite statistically significant for the bioluminescence test ($p = 0.0695$) and not statistically significant ($p = 0.2204$) for seed germination test.

To evaluate the dispersion effects, Cu-CNT complexes were exposed to the test of bacterial bioluminescence without sonication, which is a method commonly used to separate CNT aggregates in solution. The toxicity of Cu-CNT mixtures without sonication also increased in the range of 45%–56% of the control (avg. toxicity (51 ± 4.6)%). Lower toxicity, though no statistically significant differences ($p = 0.0676$), was observed compared to the results of the sets with the sonicated Cu-CNT complexes. CNTs are totally insoluble and probably one of the most biologically non-degradable man-made materials (Lam et al., 2006). The effects of the CNT particles themselves (20 and 50 mg/L) on the bioluminescence activity was also tested to support the toxicity results of the metal-CNT mixtures. No considerable toxicity on the bioluminescence activity was observed under exposure to CNT, 20–200 mg/L, showing toxicity in the range of −2% to 9% (data not shown). These results suggest that the cause of the observed toxicity of metal-CNT mixtures may be partly due to CNT particles and the observed increase in toxicity with increasing CNT percentage in the Cu-CNT mixture may not be related to the toxicity of CNTs alone.

To examine the cause of the inhibition differences, the dissolved metal ion concentrations were measured. As shown in Table 2, at 720 mg/L Cu-CNT mixtures, the concentration of Cu ions in solution were as follows: (4.2 ± 1.53), (3.6 ± 0.98), (3.4 ± 0.46), and (3.6 ± 0.50) mg Cu/L for the percentage of CNT 0, 1, 3, and 6, respectively. They showed no patterns of dissolved metal ion in solution with respect to the percentage of CNTs. This suggests that very small percentages of metals are dissolved in solution from this type of chemical, showing less than 1% for Cu-CNT mixtures, regardless of the CNT percentage. Many studies reported that the toxicity of metal oxide NPs is mainly the result of the released metal ions (Aruoja et al., 2009). Navarro et al. (2008) also reported that the toxicity of ZnO NPs may be related partly to the solubility of the NPs. CNTs may also contain varying percentages of catalyst and other impurities (biologically available degree of residual metal impurities), depending on the manufacturing process. The impact of these factors on the toxicity of CNT has not yet been determined and should be addressed in a controlled and scientific manner (Smart et al., 2006).

CNTs are harmful to living cells in culture because of their hydrophobicity and tendency to aggregate (Cui et al., 2005; Bottini et al., 2006). The presence of metal impurities can cause confusion regarding the biocompatibility, toxicity and health risks of CNTs (Zhao et al., 2008; Ge et al., 2012). For example, intracellular reactive oxygen species (ROS) were reported to be dependent on the contaminants; however, another report showed that neither purified nor non-purified CNTs containing significant amounts of iron could generate the intracellular production of superoxide radicals or nitric oxide in RAW 264.7 macrophages (Kagan et al., 2006). Ultrafine, transition-metal particles pose documented health risks upon inhalation, but their contributions to the toxicity of CNTs remain unclear due to their apparent encapsulation and immobilization by the carbon shells (Liu et al., 2007). The toxicity of a nanoparticle–copper (Cu) mixture with CNT on *Daphnia magna* were higher than that obtained from a Cu-only experiment, indicating that the mixture toxicity is additive by the presence of CNT (Kim et al., 2010). Further research will be needed to determine why the toxicity of Cu is increased in the

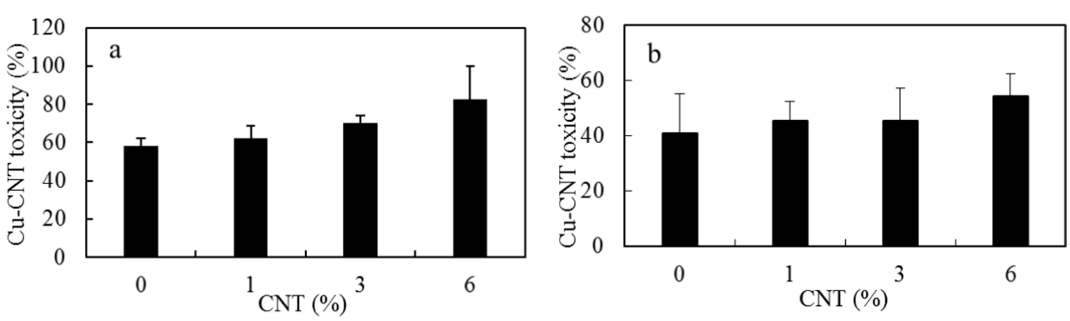

Fig. 4 – Effects of Cu-CNT mixtures on the (a) bacterial bioluminescence (720 mg/L) and (b) seed germination (10 mg/L).

Table 2 – Concentration of dissolved metal ions in a solution of metal-CNT mixtures, which were tested for bacterial bioluminescence.

Metal-CNT	Dissolved metal concentration (mg/L) in different percentage of CNT metal mixture			
	0%	1%	3%	6%
Cu-CNT	4.3 ± 1.53(00%) *	3.6 ± 0.98	3.3 ± 0.46	3.6 ± 0.50
Fe-CNT	2.1 ± 0.57	1.7 ± 0.74	3.0 ± 1.17	1.4 ± 0.52

* % of dissolved metal relative to total initial amended contents.

presence of CNTs as well as the mechanism of toxicity. ROS are chemically reactive oxygen containing molecules that are formed as byproducts of the normal metabolism of oxygen. On the other hand, the level of ROS may increase due to environmental stress, such as exposure to radiation, foreign particles *etc.* ROS may have harmful effects in cells, such as apoptosis, DNA damage, amino acid oxidation, and inactivity of enzymes (Valko et al., 2007).

2.4. Effect of Fe-CNT mixtures on the activity of bioluminescence and seed germination

Based on the determined EC_{50}, the effects of the Fe-CNT mixtures containing different percentages of CNTs on bacterial bioluminescence and seed germination were evaluated at 720 and 1850 mg/L Fe-CNT mixtures, respectively. Unlike the effects of Cu-CNT mixtures (high toxicity with increasing CNT quantity), completely opposite patterns were observed in the presence of Fe-CNT mixtures. In the case of Fe-CNT mixtures, the toxic effects decreased with increasing CNT percentage. The toxicity ranges for the Fe-CNT mixtures were as follows: 62%–73% for bacterial bioluminescence and 16%–41% for seed germination. As shown in Fig. 5, the toxicity of a Fe-CNT (6%) mixture decreased from (41 ± 12.7)% to (16 ± 9.4)% upon seed germination and from (57 ± 11.1)% to (27 ± 11.0)% upon bacterial bioluminescence in the presence of a Fe-CNT (1%) mixture, where opposite results were observed with Cu-CNT mixtures. Therefore, the toxicity with Fe-CNT (6%) mixtures were approximately 2 times higher than that of Fe-CNT (1%) on both the activity of bioluminescence and seed germination. Statistically significant and marginally significant differences were observed between the Fe-CNT mixture of 0% and 6% CNT on the bacterial bioluminescence (p = 0.0214) and seed germination (p = 0.0546), respectively.

Among several major metal residues, Fe plays a critical role in generating hydroxyl radicals, reducing the cell viability and promoting intracellular reactive oxidative species. The hydroxyl radical is highly reactive and can react with biological molecules to cause oxidative stress. The cell viability is strongly dependent on the metal residues and iron in particular, but not the tubular structure, whereas the adverse effects of the CNTs themselves on the cell viability are very limited in a certain concentration range below 80 μg/mL. CNTs are totally insoluble and probably one of the most biologically non-degradable man-made materials. Those cases proved the CNTs toxicity in the state of mixtures (Lam et al., 2006). No considerable effects by CNT itself were observed in this test. Ge et al. (2012) reported that oxidative stress induced by bioavailable metals is the dominant mechanism for CNT toxicity. For example, hydroxyl radicals can be generated through reactions that involve transition metal ions, such as iron.

Fe-CNT complexes were tested without sonication, which is a commonly used method for separating CNT aggregates in solution, to evaluate the effects of the dispersion on the bacterial bioluminescence. The toxicity by Fe-CNT mixtures was in the range of 34%–43% of the control, while those of the sonicated ones were in the range of 62%–73% of the control. Lower toxicity, though not statistical significant, was observed compared to the results of sets with sonicated Fe-CNT complexes. Therefore, dispersion in a suspension of metal-CNT complexes could partly contribute to toxicity. Dissolved Fe ion concentrations in solution were also measured to examine the cause of inhibition (Table 2). Mostly less than 3 mg/L of Fe ions (lower than 0.1% for the total Fe-CNT mixtures) were observed for the tested Fe-CNT mixtures with no general pattern, regardless of the CNT percentage. Both Cu- and Fe-CNT mixtures showed no patterns of dissolved metal ions in solution with respect to the percentage of CNT.

Xing et al. (2016) reported the possible mechanisms of metal-CNT mixtures under environmental conditions. They suggested that metal particles and CNTs repel each other when they are in close proximity due to Brownian motion, which is observed when a negatively charged surface overcomes the weak bonding caused by van der Waals forces, which are also known as agglomeration attractive forces. Dissolution and chemical transformation are also possible mechanisms that the metal

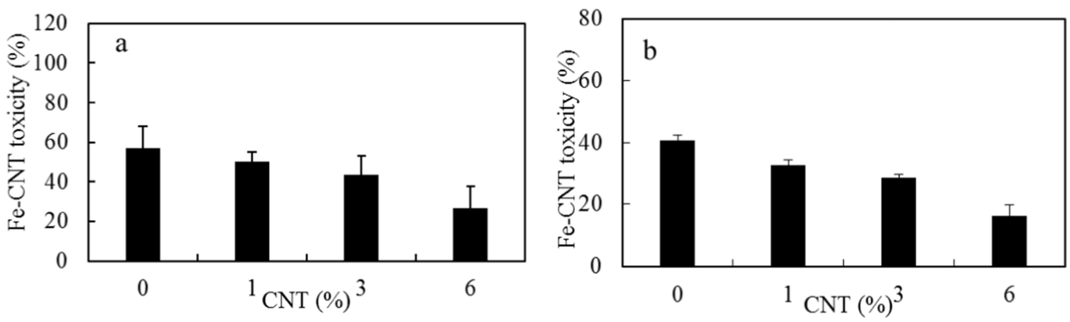

Fig. 5 – Effects of Fe mixtures with different percentages of CNTs on the (a) bacterial bioluminescence (1080 mg/L) and (b) seed germination (1850 mg/L).

particle and CNTs can undergo under environmental conditions. These processes are initially triggered by the speciation of metal particles, which are facilitated by the redox and pH conditions of natural water. When these metal ions are released from the surface, a chemical transformation based on their reactions with CNTs will occur. The presence of oxygen will lead to the formation of metal oxide species on the surface of the metal particles and increase in size when dissolved in aqueous solutions (Xing et al., 2016).

3. Conclusions

Two types of metal-CNT complexes showed different patterns of toxicity with respect to the amount of CNTs. The toxicity increased with increasing CNT content in the Cu-CNT mixtures for both organisms, while opposite results were observed with the Fe-CNT mixtures. The negative effect of CNTs themselves is very limited in a tested concentration ranges. The causes of this phenomenon are unclear at this point. On the other hand, the two metals may have different carbon solubility, chemical transformations, *etc.* in the liquid state with their own standard atomic structures and crystal lattices with the CNTs. More research will be needed to elucidate the mechanism of the toxicity of nano-mixture materials and the causes of the different patterns of toxicity with Cu- and Fe-CNT mixtures.

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