Biological compatibility of carbon nanotubes for treatment of Pollution of Nile tilapia (*Oreochromis niloticus*) by lead acetate

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Abstract: Lead (Pb^{+2}) is one of heavy metals that found in the environment and causes many adverse effects. Pb^{+2} can alter the physiological activities and causes histopathological changes of various organs in fish. Carbon nanotubes (CNTs), as a new nanomaterial, have been proven to possess great potential for removing heavy metals from water. Multi-walled carbon nanotubes (MWCNTs) were produced by CVD method. Acetylene gas was used as carbon source and Fe-Co/$CaCO_3$ as a catalyst at 600°C. The produced MWCNTs were oxidized using concentrated nitric and sulfuric acids [3:1] at 120°C, thus led to formation of oxygenous functional groups on CNTs surface. These functionalized MWCNTs were adhered to large glass sheets and can be placed in fish farm water to adsorb heavy metals, to be easily removed from water and avoid the toxicity of MWCNTS on fish. The present investigation aimed to elucidate whether the MWCNTs may elicit synergistic or antagonistic effects against acute exposure to 100 mg/L of lead acetate in the Nile tilapia *(Oreochromis niloticus*) by examining the histopathologic alterations in gills, liver and kidney. Fifty individuals of Nile tilapia with the same body weight approximately 70 g were divided into five groups (10 fish/tank). **G1:** Fish exposed to dechlorinated tap water, **G2:** Fish exposed to dechlorinated tap water containing 100 mg/L of Pb^{+2}, **G3:** Fish exposed to dechlorinated tap water containing 0.5 gm/L of MWCNTs suspended in water, **G4:** Fish exposed to dechlorinated tap water containing 100 mg/L of Pb^{+2} and 0.5gm/L of suspended MWCNTs, and **G5:** Fish exposed to dechlorinated tap water containing 100 mg/L of Pb^{+2} and 1gm/L of MWCNTs adhered by chitosan to glass sheets. Lead and/ or suspended MWCNTs caused an impact on histopathological lesions. During adsorption treatments of lead using suspended MWCNTs in water, there was much greater damage. While using immobilized MWCNTs powder fixed on glass sheets, many histopathological changes induced in other groups were decreased, but did not necessarily did not confer complete protection.

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Key words: Lead, MWCNTs, histopathology, Gill, Liver, Kidney, *Oreochromis niloticus*.

1. Introduction

Heavy metals contamination of aquatic environment has drawn increasing attention as it may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms. These metals tend to accumulate in organisms and have been found to have a variety of adverse effects on fishes. Higher concentrations of lead, cadmium and mercury were toxic to fishes (Atta *et al*., 2012). Fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Vosyliene and Jankaite, 2006 and Farombi *et al*., 2007). Fish readily absorb dissolved metals and may serve as indicators of the extent of pollution (Shukla *et al*., 2007). Tissue changes in test organisms exposed to a toxicant are a functional response of organisms that provides information on the nature of the toxicant (Mathur and Gupta, 2008). The toxic effects of heavy metals have been reviewed (Adami *et al*., 2002 and Waqar, 2006). The organisms have developed protective defense against deleterious effects of essential and non-essential heavy metals that produce degenerative changes like oxidative stress in the body (Abou EL-Naga *et al*., 2005). From bioaccumulation studies, the proportion of lead was significantly higher in different tissues of fish (Vinodhini and Narayanan, 2008). In heavy metal pollution, organs such as gills and liver have been identified as the storage sites (Gbem *et al*., 2001). However, the main sites of heavy metal uptake and accumulation are the gills and gastrointestinal tract (Patnaik *et al*., 2011).

Lead (Pb^{+2}) is a particular concern in this aspect because fish are able to bio-accumulate it in the body tissues due to reduce human food safety, especially protein source. Pb^{+2} is a non-essential metal and contemporary contaminant throughout the world. Moreover, Pb^{+2} is often used in varieties of industrial applications and products such as battery productions, chemicals, pigments and paints (Cavas, 2008). According to the previous studies, Pb^{+2} can alter the physiological activities and causes histopathological changes of various organs in fish (Jiraungkoorskul *et al*.,

2007; Lamchumchang *et al*., 2007; Singhadach *et al*., 2009; Vinodhini and Narayanan, 2008 and Yousefian and Payam, 2012). Considering that lead toxicity is currently one of the serious problems worldwide, there is still no specific, reliable and safe treatment.

Many methods including physical and chemical methods have been used to treat the pollution caused by heavy metals. Adsorption is a most common adopted method because of its simplicity and facility. Traditional adsorbents are facing great challenge because of their adsorption amount, adsorption rate or regeneration, which greatly limit the practical application. Carbon nanotubes (CNTs), as a new nanomaterial, have been proven to possess great potential for removing heavy metals, such as lead (Wang *et al*., 2007a) and copper (Stafiej and Pyrzynska, 2007) from water. In 2004 the US Environmental Protection Agency (EPA) expressed a need for the environmental applications of CNTs to be explored and remediation or treatment was identified as one of the key areas that needed to be investigated. Since then CNTs have been gaining increasing recognition for their adsorption capabilities. This is due mainly to their extremely small size, uniform pore distribution and large specific surface area (Pillay *et al*., 2009).

Multi-walled carbon nanotubes (MWCNTs) were produced by CVD method. Acetylene gas was used as carbon source and Fe-Co/$CaCO_3$ as a catalyst at 600°C. The produced MWCNTs were oxidized using concentrated nitric and sulfuric acids [3:1] at 120°C, thus led to formation of oxygenous functional groups on CNTs surface. The amount of used carbon nanotubes is very important factor to get high adsorption percentage. Adsorption capacities of the heavy metal ions increase with increasing the amount of CNTs (Li *et al*., 2003; Lu and Liu 2006 and Kabbashi *et al*., 2009), the reason is that high dose of CNTs provide more adsorption sites for attachment of heavy metals (Kabbashi *et al.,* 2009). This adsorption percentage differs either using CNTs suspended in water or fixed on glass sheets, as the later didn't have large active surface area compared to that of suspended powder (Eufinger *et al*., 2008).

CNTs were applied in powder form for heavy metal adsorption. But after adsorption process, it is difficult to completely remove powder of CNTs from treated water without centrifuging process. Separation of CNTs from treated water by filtration is difficult because the filter may be quickly blocked by CNTs. Also, adsorption of toxic substances by CNTs may enhance the toxicity of CNTs and further affect the transfer of toxic substances in the environment (Tofighy and Mohammadi, 2011). Using CNTs as adsorbent for heavy metals in fish farms must have some precautions. As it couldn't be used suspended in the treated water due to its harmful effect on fish life (Scown *et al*., 2009). This problem was solved in the present study using thin film of immobilized powder of MWCNTs fixed on glass sheets. For this purpose CNTs must be adhered with an adhesive with special features. One of the common polymers that could be used for this purpose is chitosan. Chitosan, is a polysaccharide biopolymer obtained from the deacetylation of chitin, has been widely used in medical applications because it can not only be economically processed from chitin but is also nontoxic, biocompatible, and biodegradable (Rinaudo 2006 and Mourya 2008). Using chitosan not only useful as an adhesive for CNTs, but also is known to have a good complexing ability through specific interactions of its amino groups with heavy metals from various waste waters (Rhazi *et al.,* 2002 and Elwakeel, 2010). Both carbon nanotubes and chitosan can absorb and remove heavy metals from aqueous environments (Abdel Salam *et al*., 2011), thus could help in improving the adsorption process.

Therefore, the aim of the present study is, to elucidate whether the adhesion of MWCNTs on glass sheets by chitosan may elicit synergistic or antagonistic effects against acute exposure to 100 mg/L of lead acetate in the Nile tilapia (*Oreochromis niloticus*) by examination of the histopathologic alterations in gills, liver and kidney.

2-Materials and methods:

2.1. Chemical Methods:

2.1.1. Preparation of the catalyst:

The catalyst/support (Fe-Co/$CaCO_3$) was prepared by impregnation method. Commercial $CaCO_3$ was milled for 10 hours, then iron and cobalt nitrates (Fe $(NO_3)_3.9H_2O$) and (Co $(NO_3)_2.6H_2O$) were added to the support ($CaCO_3$) with certain weight ratio (2.5: 2.5: 95) respectively. Milling was continued for 2 hours more. Few drops of distilled water were added to the produced catalyst/support mix making a paste just to ensure the homogeneity. The paste was then dried overnight at 120°C. After drying, the produced powder was grinded and handled well.

2.1.2. Preparation of MWCNTs:

For MWCNTs production, a fixed-bed reactor consisting of a furnace with a quartz tube was employed (Kathyayini *et al*., 2004). 2 gm of catalyst was uniformly spread into a small quartz boat located at the middle of the tube furnace. Temperature was gradually raised in a dynamic nitrogen flow of 70 ml/min. At 600ºC acetylene was let through with a flow rate of 10 ml/min. After an hour of the reaction time, the flow of acetylene was stopped and the furnace was allowed to cool to room temperature in a nitrogen atmosphere (Kathyayini *et al*., 2004). The produced MWCNTs were collected for purification and characterization

2.1.3. Functionalization and characterization of produced MWCNTs:

Specific amount of produced MWCNTs were added to a mixture of concentrated nitric acid /sulfuric acid [3:1] respectively. The mixture was refluxed in oil bath for 6 hrs at 120°C, cooled to room temperature, diluted with distilled water and then filtered through a filter paper (3µm porosity). This washing operation was repeated several times and then the mixture was dried at 100°C (Wang *et al*., 2007a and Tofighy and Mohammadi, 2011). The functionalized MWCNTs were characterized by TEM microscopy and the produced functional groups were investigated by using Fourier transform infrared spectroscopy.

2.1.4. Optimizing the amount of MWCNTs:

Several weights of MWCNTs 0.1, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5 and 0.6 gm were added to 100 ml solutions of Pb (II) ion with concentration 100 mg/L at pH 7. The prepared samples were shaken with mechanical shaker at 25°C for 10 hrs. The MWCNTs were removed by filtration with Whatman filter paper. The concentration of heavy metal ions before and after adsorption was determined using atomic adsorption mass spectrometer. For accurate adsorption results, all the adsorption data were analyzed three times and the results were averaged. The amount of adsorbed metal was calculated using the following equation (Kandah and Meunier, 2007):

$$q_e = \frac{(c_o - c_c)v}{w}$$

Where:
q_e: is the amount of adsorbed metal (mg/g)
C_0: concentration of initial solution (mg/L)
C_e: concentration after adsorption (mg/L)
V: volume of initial solution (l)
W: amount of MWCNTs used (gm).
The adsorption percentage was calculated by this equation (Gao *et al*., 2009):
where: $C_\circ$ is the initial concentration
C_i is the equilibrium concentration.

2.1.5. Preparation of chitosan thin layer on glass sheets:

Five pieces of glass sheets (10 cm X 20 cm) were used to form chitosan layers on their surfaces. 2gm of chitosan powder were added into 100 ml of distilled water with continuous stirring on hot until the solution became homogenous. Then 2gm of acetic acid were added to the solution with continuous stirring till the liquid became homogenous and clear, that took about 30-60 min (Annala, 2007). The viscosity of chitosan solution is quite high, as syrup. Small amount of solution were taken with spatula and put on each glass sheet to form a thin layer of chitosan, which were used as an adhesive for carbon nanotubes on these glass sheets.

2.1.6. Preparation of carbon nanotubes thin films on glass sheets:

The prepared glass sheets with chitosan were used for this purpose, as 20 gm of functionalized MWCNTs were spread onto chitosan thin layers. The excess of MWCNTs were removed before using. The glass sheets were kept in room temperature till drying then were put in the bottom of the treated fish aquarium.

Three samples of 100 ml lead acetate with concentration 100 mg/L at pH 7 were used, the first has 0.5gm of suspended MWCNTs in water, the other two samples were glass sheets have (0.5 and 1gm) respectively of immobilized powder of MWCNTs fixed on glass sheets. The prepared samples were shaken with mechanical shaker at 25°C for 10 hrs. After shaking time, the suspended MWCNTs were filtered with Whatman filter paper while the other two samples, the glass sheets, were removed from their solutions. The concentration of Pb^{+2}ions before and after adsorption was determined using atomic adsorption mass spectrometer. For accurate adsorption results, all the adsorption data were analyzed three times and the results were averaged. The amount of adsorbed metal and the adsorption percentage were calculated using the previous two equations.

2.2. Biological Methods:

Healthy fish of Nile tilapia (*Oreochromis niloticus*) weighing approximately 70 g/fish were collected from agriculture farm, Faculty of agriculture, Fayoum Governorate, Egypt. Fish were transferred to the laboratory and acclimated for 2 weeks in large tanks with well aerated dechlorinated tap water (25 ± 10C). The pH was 7.3 ± 0.2 and oxygen content was 7.2 mg/l that was maintained constant using air pump. All the studied fishes were individually examined to make sure that they are free from any skin lesions or apparent disease symptoms.

2.2.1. Experimental Design:

Fifty individuals of Nile tilapia with the same body weight approximately 70 g were divided into five groups and stocked into five well aerated glass aquaria 120 L each (10 fish/ tank). Fish of the different studied groups were exposed to lead acetate and MWCNTs for 96 hours as follows:

$$\text{Adsorption percentage}\,\% = \frac{(c_o - c_i)}{c_o} \times 100\%$$

Group 1: Fish exposed to dechlorinated tap water.
Group 2: Fish exposed to dechlorinated tap water containing 100 mg/L Pb^{+2}, which correspond to 50% of

the 96h LC50 according to (Kosia *et al*., 2011) and as application for the present chemical study.
Group 3: Fish exposed to dechlorinated tap water containing 0.5 gm/L MWCNTs suspended in water.
Group 4: Fish exposed to dechlorinated tap water containing 100 mg/L Pb^{+2} and 0.5 gm/L of suspended MWCNTs.
Group 5: Fish exposed to dechlorinated tap water containing 100 mg/L Pb^{+2} and 1gm/L MWCNTs adhered by chitosan to glass sheets.

2.2.2. Histological Preparations:

The control and exposed fish in all groups were rapidly dissected at the end of the experiment from the ventral line beginning with the anal papilla till the gill operculum. Two lobes of the liver were recognized and for this investigation, always pieces of the left lobe of the liver were excised. Also pieces of the kidney and the second gill arch from the right bronchial chamber of each fish were removed. These specimens were put directly in neutral buffered formalin then dehydrated, cleared and embedded in paraplast plus (m.p.56-58 C°). The gills were oriented with their middle hemibranchs placed downwards. Paraffin sections 4-6 µm thick were then cut with a rotary microtome and stained with Haematoxyline and eosin (H & E) according to (Bancroft and Gamble, 2002) for studying the general morphology of gill, liver and kidney.

3-Results

3.1. Chemical observations of MWCNTs:

Figure 1 (A&B) show TEM images of the oxidized MWCNTs. Fig. 1A indicated that well graphitized walls without any remarkable coverage of other materials were observed clearly. Fig. 1B showed that walls of MWCNT were clearly observed with thickness about 5 nm with inner and outer diameter of the tube about 3 and 19 nm respectively.

High purity oxidized MWCNTs were produced which is known as functionalized MWCNTs due to carboxylic groups attached to its surface after acid treatment. Fig. 2 showed Fourier transform infrared spectra in the range 500–4000 cm^{-1} for the oxidized MWCNTs sample. Spectra showed a band around 3438 cm^{-1}, 1462 cm^{-1}, 2916 cm^{-1}, 2850 cm^{-1}, 1575 cm^{-1}, 1704 cm^{-1} and 1134 cm^{-1}.

Figure 3 showed the effect of MWCNTs dose on the adsorption percentage. The adsorption percent (%) of lead ions onto MWCNTs reached sharply to 13.63, 23.55, 39.13, 50.96, 72.23, 73.1 and 73 as the MWCNTs weight increased from 0.1, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5 to 0.6gm respectively. Therefore, the increase of MWCNTs dose can obviously increase the adsorption percentage of Pb^{+2} ions.

Table 1 shows the difference between MWCNTs as suspension and thin film on glass sheets and its effect on adsorption percentage of Pb^{+2} ions. From the table found that the adsorption mean percentage % in case of suspension of 0.5 gm MWCNTs was 98, while in case of 0.5 and 1gm of thin film of MWCNTs fixed on glass sheets were 80 and 95 respectively. This result shows that the adsorption mean percentage % for the later two samples is lower than that of MWCNTs suspended in treated water.

Table 1: Effect of CNTs as suspension and thin film on glass sheets on mean adsorption percentage of Pb^{+2}

Amount of CNTs	Mean adsorption percentage %
0.5 gm of CNTs suspended in water	98 %
0.5 gm of CNTs spread on glass sheet	80 %
1gm of CNTs spread on glass sheet	95 %

3.2. Biological observations:

3.2.1. Histopathological observations of gills:

The gill filaments are long thread-like structures covered by a stratified squamous epithelium, with a central core of connective tissue. The entire surface of the gill filaments is bound by a respiratory epithelium which bears leafy structures, known as the secondary gill lamellae. At the same time, the respiratory lamellae are covered by a flattened epithelium, one layer thick (Fig.4 A). The distinct histopathological change in gills of fish exposed to lead acetate was represented by hypertrophy and destruction of lamellar architecture and hyperplasia that resulted in the fusion of many lamellae. The epithelial covering of the gill filaments was hyperplastic and edematous. In some areas, the hyperplastic interlamellar epithelial cells reached the tips of the lamellae causing complete fusion of the gill lamellae (Fig.4 B). In fish exposed to MWCNTs suspended in water, drooping and shortening of some of the secondary lamellae were observed. Further epithelial cell hyperplasia of the secondary lamellae resulted in adhesion of the lifting respiratory epithelium of the lamellae. Epithelial desquamation (lifting) was observed of most respiratory lamellae (Fig. 4 C). In fish exposed to lead acetate and suspended MWCNTs shortening of the secondary lamellae and hyperplasia of the interlamellar epithelial cells of the gill filaments starting at the bases of the secondary lamellae and extending towards their tips causing complete fusion of the secondary lamellae. Epithelial desquamation (lifting) was observed from the base towards the tips of most respiratory lamellae and may lead to rupture of the secondary lamellae with complete necrotic areas (Fig.4 D). In fish exposed to lead acetate and adhered MWCNTs, The gill filaments were more or less similar

to those of control group. The gill filaments were also intact with slight drooping but showed none of the other signs of lamellar epithelial desquamation, interlamellar hyperplasia or lamellar fusion observed in fish exposed to lead acetate and/ or suspended MWCNTs (Fig. 4 E).

3.2.2. Histopathological Observations of liver:

In control group the liver tissue generally exhibited a normal architecture with polygonal shaped hepatocytes, having a large spherical nucleus with variable amount of dispersed and peripheral heterochromatin. Hepatocytes were located among blood capillaries called sinusoids forming cord-like structure known as hepatic cell cords. The hepatopancreatic acini circumscribing portal veins are of variable sizes. The cells of this hepatopancreas are large columnar pseudostratified having one or two basal nuclei (Fig. 5A). In the lead acetate-exposed group degenerative effects were evident among hepatocytes such as disarrangement of hepatic plates and contact loss between hepatocytes. The hepatocytes exhibited focal necrosis resulting in complete disintegration of cellular components as evidenced by the presence of darkly stained eosinophilic debris (Fig. 5B). Sections from fish liver from aquaria containing suspended MWCNTs showed several changes. The liver cells were degenerated; the normal architecture of liver was markedly disorganized. In addition pyknotic nuclei, dilated sinusoids and degeneration of hepatopancreas were observed (Fig.**5**C). Liver of fish exposed to lead acetate and suspended MWCNTs revealed varying degrees of histopathological alterations due to damaging of cell structure. Large clear vacuoles were found among these hepatocytes. Large lysed or necrotic areas of liver parenchyma, hepatocytes with large intracellular and intercellular vacuoles were evident among the liver parenchyma (Figs. 5 D). Fish exposed to lead acetate with adhered MWCNTs showed more or less normal liver architecture. Some hepatocytes had better defined boundaries and the hepatopancreas appeared nearly normal than the previous groups (Fig. 5E).

3.2.3. Histopathological Observations of kidney:

The control kidney was composed of numerous renal corpuscles with well developed glomeruli and a system of tubules. The proximal segment was covered by tall columnar epithelial cells with basal nuclei and brush border located along the cell apices. The distal segment was lined with large, relatively clear columnar epithelial cells with central nuclei and the brush border was reduced or absent. The collecting duct was larger in diameter than the distal segment, containing columnar epithelial cells with basal nuclei and no brush border (Fig.6 A). After lead exposure, kidney sections were showed severe damage and disorganization of tubules. Reduction in glomerulus, resulting in dilation of Bowman's space was observed. Severe damage and disorganization of the tubules were also observed. Degeneration of some epithelial cells lining the renal tubules in addition to tubular narrowing was also found in the renal tubules (Fig. 6 B). After MWCNTs exposure, renal tissues were shown the most conspicuous alterations. Degeneration of some epithelial cells lining the renal tubules and areas of dissolution in the cortex were observed. Tubular narrowing and hyaline droplet were also found in renal tubules (Fig. 6 C). The most important changes found in the kidney of lead and suspended MWCNTs exposed fish was reduction in glomerulus, resulting in dilation of Bowman's space. Degeneration of most epithelial cells lining the renal tubules and focal necrosis resulted in complete disintegration of cellular components in addition to hemorrhage among the renal parenchyma (Figs. 6D). Exposure of fish to lead acetate and adhered MWCNTs showed nearly normal renal corpuscles, glomerulus, Bowman's space and renal tubules (Fig.6 E).

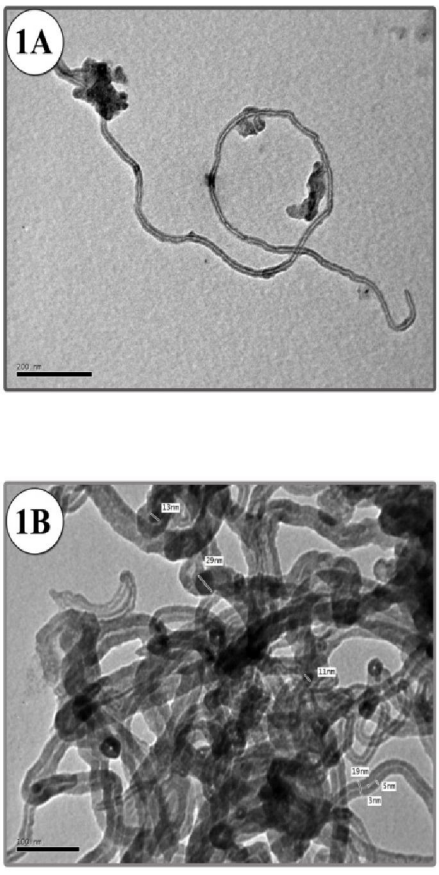

Figure 1 (A, B): Transmission Electron Microscopic (TEM) photos of MWCNTs synthesized at 600 ^{o}C and oxidized in concentrated acid for 6 hrs.

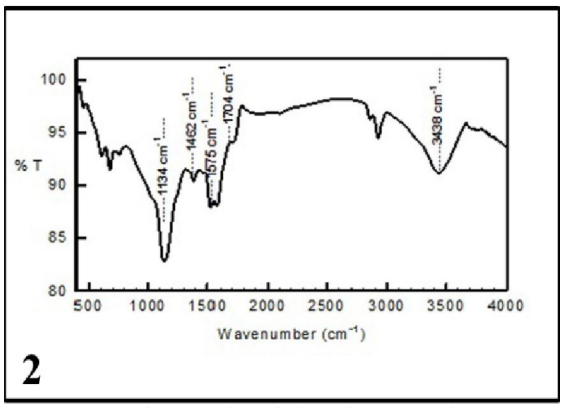

Figure 2: Fourier transform infrared spectra of MWCNTs synthesized at 600 ^{o}C and then oxidized in concentrated acid for 6 hrs.

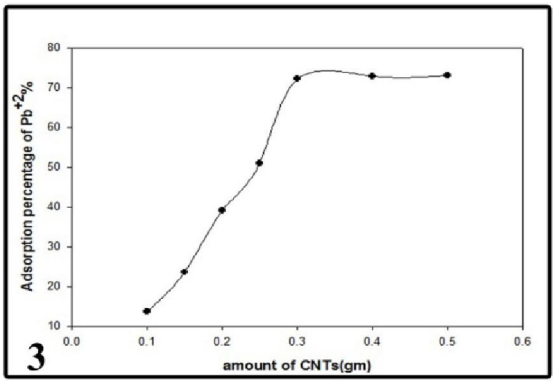

Figure 3: Shows the effect of the MWCNTs on the adsorption of Pb^{+2}.

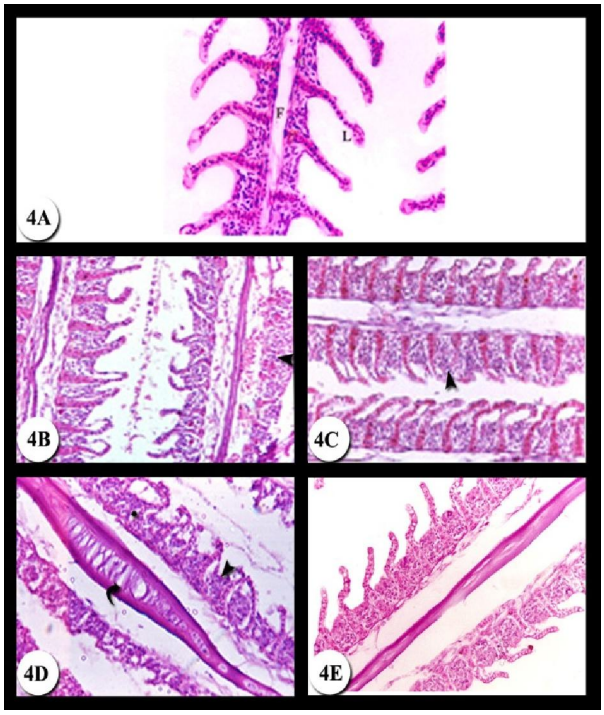

Figure 4: Sections in gills of *Oreochromis niloticus* showing **A)** control filament (F) and secondary lamellae (L) arising from these, parallel to each other and perpendicular to the filament axis (H and E, X 400). **B)** fish exposed to lead showing fusion of the secondary lamellae (head arrow) (H and E, X 200). **C)** fish exposed to MWCNTs showing complete fusion of secondary lamellae (head arrow) (H and E, X 200). **D)** fish exposed to lead and suspended MWCNTs showing hyperplasia starting at the bases of the filaments and extending towards their tips with complete fusion of the secondary lamllae (arrowhead) and separation of epithelial cells forming edema (asterisk) and deformed cartilaginous skelton (bent arrow) (H and E, X 400). **E)** fish exposed to lead and adhesive MWCNTs showing, no detectable changes at the epithelium of the gill filaments and drooping of the secondary lamellae were slight (H and E, X 200).

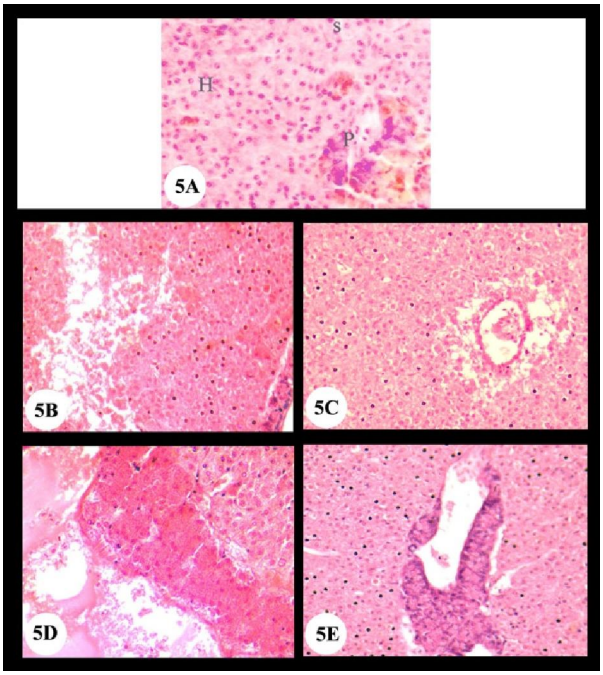

Figure 5: Sections in liver of *Oreochromis niloticus* of showing **A):** normal hepatocytes (H) with sinusoid and hepatopancreas (P) (H and E; X 400). **B):** lead exposed group with necrotic areas of liver parenchyma (H and E; X 400). **C)** MWCNTs exposed group with necrotic areas of liver parenchyma and hepatopancreas (H and E; X 400). **D):** lead and suspended MWCNTs exposed group showing focal necrosis of hepatic tissue (H and E; X 400). **E)** lead and adhered MWCNTs exposed group showing nearly normal hepatic architecture (H and E; X 400).

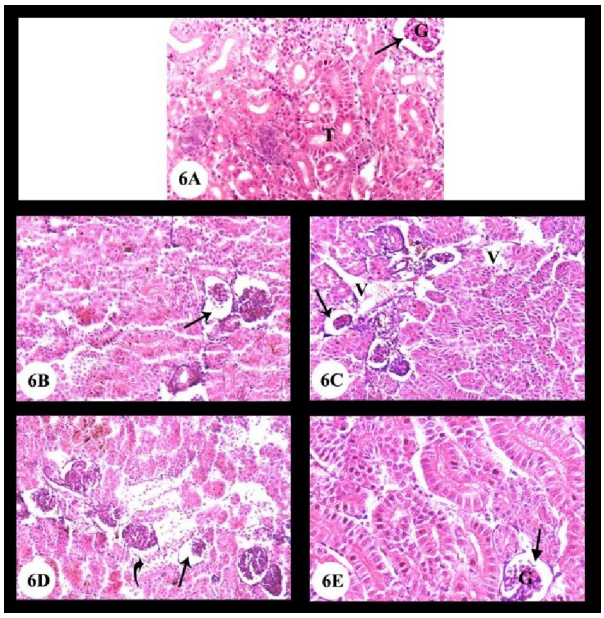

Figure 6: Sections in kidney of *Oreochromis niloticus* showing **A):** normal renal corpuscle, glomerulus (G), Bowman's space (arrow) and renal tubules (T) in control group (H and E; X 400). **B):** Fish exposed to Lead acetate group showing renal tubule cells with degeneration and dilation of Bowman's space (arrow) (H and E; X 200). **C):** MWCNTs exposed group showing vacular degeneration (V) and dilation of Bowman's space (arrow) (H and E; X 200). **D)** lead acetate and suspended MWCNTs exposed group showing shrinkage in the glomeruli, dilation of Bowman's space (arrow), degenerated tubules and hemorrhage among the renal parenchyma (H and E; X 200). **E):** Lead acetate and adhered MWCNTs group showing nearly normal renal corpuscles, glomerulus (G), Bowman's space (arrow) and renal tubules (H and E; X 400).

4-Discussion

In the present study, the prepared MWCNTs were functionalized using chemical oxidation method (Wang *et al*. 2007a and Tofighy and Mohammadi, 2011). The effect of functionalization on MWCNTs surface was examined by TEM (Wang *et al*., 2007 a, b). This step is very important as the functional groups were formed on its surface and they were investigated by using Fourier transform infrared spectroscopy (Hu *et al*., 2009 and Yang *et al*., 2009). These functional groups could react with the metal ion by chemical interaction causing its adsorption on CNTs surface (Lu *et al*., 2006 and Lu and Liu, 2006).

High purity oxidized MWCNTs were produced which is known as functionalized MWCNTs due to carboxylic groups attached to its surface after acid treatment. Fig. 2 showed Fourier transform infrared spectra in the range 500–4000 cm^{-1} for the oxidized MWCNTs sample. Spectra showed a band around 3438 cm^{-1} which can be attributed to the hydroxyl group (-OH) (Yang *et al*., 2009). Band around 1462 cm^{-1} due to (C=O) bond (Yang *et al*., 2009). Bands around 2916 and 2850 cm^{-1} are due to asymmetric and symmetric stretching of (C–H) bond. The band around 1575 cm−1 can be attributed to asymmetric carboxylate anion stretch mode. The band around 1704 cm^{-1} can be attributed to either ester or carboxylic groups (Yang *et al*., 2009). The appearance of a band around 1134 cm^{-1} corresponds to carboxylic-OH group (Lu *et al*., 2008).

Figure 3 shows The effect of MWCNTs dose on the adsorption percentage. The adsorption percentage of Pb^{+2} increased with increasing the amount of CNTs this is in accordance to (Li *et al.,* 2003; Lu and Liu, 2006 and Kabbashi *et al*., 2009).

From table 1, adsorption mean percentage for both samples of thin films of MWCNTs fixed on glass sheets were lower than that of MWCNTs suspended in treated water. That is due to low surface area in case of thin film of MWCNTs (Eufinger *et al.,* 2008). As a result, low adsorption sites would be available for metal ions, hence, the adsorption percentage will decrease. The amount of MWCNTs fixed on glass sheets were duplicated to get higher surface area and to get higher adsorption percentage as in case of 0.5gm/L MWCNTs suspended in water.

Histopathological alterations can be used as indicators of the effects of various pollutants on the organism including fish, and reflection of the overall health of the entire pollution. (Mohamed, 2009) reported that the exposure of fish to pollutants, that is agricultural and industrial chemicals, were resulted in several pathological changes in different tissues of fish. Nile tilapia; *Oreochromis niloticus* was selected for this study as it is considered as an important fish cultured in Egypt. Its rapid growth rate under tropical climates has led to widespread distribution.

The present study examined the histopathological changes of lead acetate exposure in control, fish exposed to Pb and /or MWCNTs suspended in water or adhered to glass sheets as an application for the chemical part of the present study. The results presented here revealed that lead exposure resulted in histopathological changes in gills, liver and kidney of *O. niloticus* and no one of the exposed fish could escape the toxic effect of lead. Gills showed edema of the primary lamellae, hyperplasia, fusion and focal desquamation of the epithelial lining of the secondary lamellae. The observed changes in fish gills were generally attributed to toxic effects of lead. Similar observations were reported due to exposure of other freshwater fish to lead (Palaniappan, 2008 and Mobarak and Sharaf, 2011), copper (Park and Heo, 2009 and Kosai, 2009) and other metals (Velmurugan *et al*., 2007 a, b; Velmurugan *et al*., 2009; Flores-Lopes and Thomaz, 2011).

The histopathological alterations attributed to heavy metals exposure resulted in respiratory, osmoregulatory and circulatory impairment. These

findings were demonstrated by (Fernandes *et al.,* 2008). Moreover, (Alvarado *et al*., 2006) reported that, the dramatic increase of chloride cells in the gills that produces epithelial thickening of the filament epithelium enhances migration of chloride cells up to the edge of the secondary lamellae and provokes the hypertrophy and fusion of secondary lamellae. These could be considered as unspecific biomarker responses of heavy metals exposure and disturbed health of fish.

According to (Kaoud and El-Dahshan, 2010), the edema of the gill epithelium is one of the main structural changes caused by the exposure to heavy metals. The present results confirm this lesion of heavy metals exposure. These alterations have been reported for other species exposed to heavy metals (Thophon *et al.,* 2003) and sometimes referred as a first sign of pathology (Thophon *et al.,* 2003). Cellular proliferation in the gill epithelium is also observed in fish exposed to different pollutants as described by (Thophon *et al.,* 2003). Lifting and hyperplasia of the gill epithelium could serve as a defense function, as these alterations increase the distance across which waterborne irritants must diffuse to reach the bloodstream. Lamellar fusion could be protective once it reduces the amount of vulnerable gill surface area (Kaoud and El-Dahshan, 2010).

The organ most associated with the detoxification and biotransformation process is the liver, and due to its function, position and blood supply (Van der Oost *et al*., 2003) it is also one of the organs most affected by contaminants in the water (Alwan and Hadi, 2012). In the present study the liver showed degeneration of the hepatocytes and hepatopancreas and nuclear pyknosis in the majority of hepatic cells. Similar alterations were observed in several species of fish treated with heavy metals (Mobarak and Sharaf, 2011 and Deore and Wagh, 2012). Anomalies such as irregular shaped hepatocytes, cytoplasmic vacuolation were also described in the siluriform *Corydoras paleatus* contaminated by organophosphate pesticides (Fanta *et al*., 2003). Also (Pacheco and Santos, 2002) described increased vacuolization of the hepatocytes as a signal of degenerative process that suggests metabolic damage, possibly related to exposure to contaminated water. These alterations are more severe and have been associated with the exposure of fishes to contamination by metals, such as copper (Paris-Palacios *et al*., 2000) and lead (Deore and wagh, 2012).

Liver of fish is sensitive to environmental contaminants because many contaminants tend to accumulate in the liver and exposing it to a much higher levels than in the environment, or in other organs (Oliveira-Ribeiro *et al*., 2002).

In fish, as in higher vertebrates, the kidney performs an important function to maintain the homeostasis. It is responsible for selective reabsorbtion, which helps in maintaining volume and pH of blood and body fluids and erythropoieses (Iqbal *et al*., 2004). The kidney is one of the first organs to be affected by contaminants in the water (Thophon *et al*., 2003 and Mela *et al*., 2007). In the present study, histopathological alterations in the kidney of fish exposed to lead were disorganization of tubules, degeneration of some epithelial cells lining the renal tubules, reduction in glomerulus and dilatation of Bowman's space. Similar alterations in kidney of Tilapia were observed in several species of fish exposed to heavy metals and these alterations were described by (Oliveira-Ribeiro *et al*., 2002; Jiraungkoorskul *et al*., 2003; Thophon *et al*., 2003; Gupta and Srivastava, 2006 and Alwan and Handi, 2012).

Most common alterations found in the kidney of fishes exposed to water contamination are tubule degeneration and changes in the corpuscle (Takashima and Hibiya, 1995). Exposure to metals frequently causes alterations in the tubules and glomerulus, such as was described by (Thophon *et al*., 2003) for the perch (*Lates calcarifer*) exposed to cadmium; (Handy and Penrice, 1993) found swollen Bowman´s capsule cells and melanomacrophages in the kidney of trout (*Salmo trutta*) and tilapia (*Oreochromis mossambicus*) exposed to mercuric chloride. Similar alterations were found in fishes exposed to organic contaminants (Veiga *et al*., 2002) and mixed environmental contaminants (Schwaiger *et al*., 1997 and Pacheco and Santos, 2002). In more severe cases, the degenerative process can lead to tissue necrosis (Camargo and Martinez, 2007). Lead was found to inhibit the impulse conductivity by inhibiting the activities of monoamine oxidase and acetylcholine esterase to cause pathological changes in tissue and organs (Kaoud and El-dahshan, 2010).

Mammalian studies have raised concerns about the toxicity of carbon nanotubes (CNTs), but there is very limited data on ecotoxicity to aquatic life. The results presented here revealed that MWCNTs exposure resulted in various histopathological changes in gills, liver and kidney. This result could be explained by the fact that CNTs accumulates selectively in gills (the principal site of gaseous exchange, body fluids pH regulation and nitrogenous waste excretion) of fresh water fish that may lead to impairment of gill functions as respiration, osmotic and ionic regulation and excretion of nitrogenous wastes. Impairment of these functions will certainly affect structures and functions of different fish organs and eventually may lead to the observed histopathological changes.

Although little is known about the mechanisms of nanomaterial toxicity, other researchers have provided evidence for nanoparticle-mediated production of reactive oxygen species and generation of oxidative stress as a possible mechanism of toxicity (Zhu *et al*., 2007; Zhu *et al*., 2009 and Zhang *et al.,* 2010) for carbonaceous nanoparticles (i.e., fullerenes, fullerols,

and carbon nanotubes). Several studies demonstrated toxic effects of engineered nanoparticles such as inflammation (Grassian *et al*., 2007), cytoskeletal and membrane changes (Shvedova *et al*., 2003), oxidative stress (Limbach *et al*., 2007), apoptosis (Park *et al*., 2008) and changes in gene expression (Fujita *et al*., 2009).

In exposure to carbon nanomaterials, Yousefian and Payam (2012) reported signs of gill irritation and mucus secretion during exposure to single wall carbon nanotubes (SWCNTs). Also fish exposed to low or high SWCNT concentration shows different appearance related to dosage of carbon treatment (Yousefian and Payam, 2012). Fish gill surfaces may be more susceptible to irritation by nanoparticles (NPs) of a defined size range, or specific sizes or shapes; and it may be more difficult for fish to dislodge some shapes or sizes of carbon nanomaterials from the cell surface.

The addition of immobilized MWCNTS adhered to glass sheets, in the present study, to water containing lead caused a decrease in histopathological changes induced by lead. The present results agree with (Wang *et al*., 2007 b), who reported that CNTs possess great potential for removing heavy metals, such as lead from aqueous solution (no fish).

Smith *et al*. (2007) described the first detailed report on the toxicity of carbon nanotubes (CNTs) to rainbow trout, (*Oncorhynchus mykiss*) using a body systems approach. The authors reported that CNTs exposure caused a dose-dependent rise in ventilation rate, gill pathologies (oedema, altered mucocytes, hyperplasia), and mucus secretion with CNTs precipitation on the gill mucus. Liver cells exposed to CNTs showed condensed nuclear bodies (apoptotic bodies) and cells in abnormal nuclear division (Smith *et al*., 2007).

The present histopathological study showed that fish exposed to lead and/or suspended MWCNTs showed much greater damage in comparison with fish exposed to lead and fixed CNTs (adhered to glass sheets). The nanoparticles (NP) is acting as a "delivery vehicle" for the metal ions, and when the NP sticks to gill surface (for example) it could release locally high concentrations of metal ions, or provide a sustained slow release of metal ions onto the epithelia. There is also evidence of "delivery vehicle" effects when the metal is present as a co-contaminant with a NP. This may be related to the ability of metals to adsorp on the surface of some negatively charged NPs (Handy *et al*., 2008). For example, (Zhang *et al*., 2007) exposed carp to TiO_2 nanoparticles and found that the fish accumulated 146% more Cd in the presence of the TiO_2 nanoparticles compared to when fish were exposed to Cd alone. Similarly, carp exposed to the metalloid, arsenic (As (V)), in the presence of TiO_2 nanoparticles accumulated 132% more than fish exposed to an equal concentration of As only (Sun *et al.*, 2007).

In the present study lead adsorbed on MWCNTs surface, and adhesion of MWCNTs by chitosan on glass sheets may prevent MWCNTs to act as a delivery vehicle for lead thus prevent the lead Toxicity.

5-Conclusion:

The present findings demonstrated that both lead and/or suspended MWCNTs had adverse effects on gill , liver and kidney histology. On the other hand, it was observed that adhesion of MWCNTs to glass sheets by chitosan improved the histopathological changes induced by lead in some degree. It can be concluded that adhesive MWCNTs has a protective effect against lead adverse effects by adsorbing (scavenging) suspended lead on their surfaces and histopathology can be used as bioassays for monitoring pollution in aquatic medium.

MWCNTs adhered to large glass sheets can be placed in fish farm water to adsorb heavy metals from the water, to be easily removed from water and to avoid the toxicity of MWCNTS on fish.

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