**Egyptian Journal of Chemistry**

**http://ejchem.journals.ekb.eg/**

# Carbon nanotube-based filters for heavy metal removal and their effect on the aquatic conditions of freshwater bivalves in River Nile, Egypt

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**Abstract**

Chemically functionalized multi-walled carbon nanotubes (F-MWNTs) offer a lot of potential as a novel effective filter due to their unique qualities such as chemical stability, thermal permanence, and high surface area. This study aims to investigate the efficiency of F-MWNTs filters to improve the aquatic conditions for the freshwater bivalve, Mutela singularis (*M. singularis*). It was shown that the removal performance of F-MWNTs filters for the heavy metals Co, Cu, Fe, Pb, and Zn is favorable at high pH, while Cd, Ni, and Cr are removed at low pH from aqueous solutions. F-MWNTs-based filters removed 99.8% of copper with a 5 µg/L concentration at pH=10. The mechanisms underlying F-MWNT filtration as a feature of pH were discussed. The histological and biochemical composition of *M. singularis* was studied before and after the use of F-MWNT filters. *M. singularis* samples in purified conditions had a high standard of structure, as well as a large increase in carbohydrate, lipid, and protein content, for example, the protein increased by 45%. The advancement of wastewater treatment with F-MWNT filters has high expectations. Our research contributes significantly to water treatment applications, and to the potential feeding fields.

**Keywords:** Heavy metal removal; Aquatic conditions enhancement; Carbon nanotube filters; River Nile treatment

## 1. Introduction

Molluscs are highly delicious seafood and they are also a very good source for biomedically important products [1]. The name "shellfish" refers to a wide range of species, including bivalves such as clams, quahogs, oysters, mussels, scallops, crabs and shrimp [2]. Human activities that have been done without appropriate control may release heavy metals pollution into rivers and the environment [3]. Bacteria can be found in abundance in watery environments. Bacterial infections of mollusks have been commonly recorded [4]. Because many mollusks, particularly freshwater bivalves, amass and harbor a large commensal zoo of bacteria, it can be difficult to detect bacteria that are considered harmful [5]. *Staphylococcus aureus* (*S.aureus*) and *Vibrio alginolyticus* (*V. alginolyticus*), for example, were the most common seafood pathogens that caused skin infections, respiratory tract infections, intestinal tract infections, and bloodstream infections [6]. Sea foods are susceptible to bacterial infection, posing a health concern to humans [7]. Since bivalves are filter feeders, the concentration of toxins is more than that of the neighboring sea water. Because raw or minimally cooked shellfish can be consumed, these contaminants, particularly microbiological contamination, may cause human sickness [8]. The source of the shellfish should be verified to reduce the risk, and proper treatment after processing will improve the quality.

The Nile River is plagued by ecological and public health issues as a result of impurities that degrade water quality and disrupt ecosystem equilibrium [9]. Heavy metals can enter the aquatic ecosystem through a variety of natural and anthropogenic sources, including as industrial or domestic sewage, storm runoff, landfill/dumpsite leaching, and

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**Receive Date: 12 September 2021, Revise Date: 30 September 2021, Accept Date: 04 October 2021**
**DOI: 10.21608/EJCHEM.2021.95658.4490**

atmospheric deposits [10]. The concentrations of elements in mollusks at the same region fluctuate between individuals [11]. While there is a paucity of data on contaminated areas in tropical areas, studies on monitoring pollution in the surrounding area of freshwater lakes based on several indicator species have been noted [12].

Mollusks in lakes and ponds are significant in biological diversity and health of the ecosystem [12]. Environmental contaminants can have direct harmful effects on tissues or cells, as well as alterations in homeostatic mechanisms, which can have an impact on immunity [13]. The amount of protein in animal tissues affects animal metabolism and reveals heavy metal effects. Toxic substances primarily interact with proteins, altering physiological functions and putting lives at risk in a variety of ways. Protein serves as the animal's enzyme, hormone and fundamental structural component. Because of its toxicity to freshwater life, plants, animals, humans, and the environment, heavy metal poisons have long been a source of worry [14].

The presence of a disproportionate amount of heavy metals in an aqueous environment, such as arsenic, chromium, cadmium, arsenic, lead, copper, nickel and, zinc might be of great concern due to their toxicity and carcinogenicity, which can harm a variety of human body functions [10]. Nanotechnology may hold the key to ensuring a reliable supply of drinking water in areas of the world plagued by periodic droughts and water contamination [15]. Nanotechnology is used in various fields such as water treatment [16, 17], biomedical applications [18], and many others. In recent years, carbon nanotubes (CNTs) have made considerable improvements in the field of water purification [15].

CNTs' ability to remove heavy metals is determined by their shape, which is determined by the type of treatment employed to improve filtration efficiency [19]. Multi-walled carbon nanotubes (MWNT) have previously been used to extract metal ions such as zinc, copper, cadmium, silver, nickel, and others [20]. The performance of MWCNT-based filters is determined by the nanotubes' physicochemical properties, which vary depending on the type of modification [21, 22].

Although there have been numerous studies on the use of MWNTs as heavy metal removal filters, their results have yet to be thoroughly investigated, especially for the enhancement of aquatic media for mollusks. This study claims that F-MWNTs filters increase the filtration performance of heavy metals. Scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), and FTIR spectrometer were used to investigate the functionalization effects on the CNT filter. The influence of pH on the effectiveness of removal has been investigated. In Abu Hummus, Egypt's River Nile, heavy metal levels in water, sediment, and *M. singularis* flesh were measured. Our investigations explore the suitability of *M. singularis* as a bio-marker that might be used to track the pollution of heavy metals in the Nile River and to demonstrate the impact of contaminants on the biochemical products (protein, lipid and Carbohydrates).

## 2. Methods

### ***M. singularis*** sample preparation and bacteriological investigations

M. singularis, a sampling bivalve, was collected from its natural beds in Abu Hummus El Behira, Egypt, during the summer season of 2020 (June-September) and morphologically studied to identify its status (health, unhealthy). The Agriculture Research Center (ARC), Faculty of Agriculture, Cairo University Egypt, prepared heavy metal samples to detect trace metal concentrations (Cd, Co, Cr, Cu, Fe, Ni, Pb, and Zn). The heavy metal stock solution was made by dissolving the metal salts in distilled water and diluting the solution to the desired concentration. Buffer solutions are used to modify the pH of the solutions. Mussel samples were weighed and digested with $HNO_3$ in Teflon digestion containers after being removed from their shells. ION-selective electrode AVL was used to assess wet digested samples that had been diluted with deionized distilled water. The results were represented as a wet weight of μg/g. Three replicate measurements for the sample were used to verify the analytical process. Gill swabs from captured mussels and swabs from water samples were inserted right away in the nutrient agar transfer media. Each specimen was grown on blood agar and nutrient agar plates. In these media, the resulting colonies were subcultured on Mac Conkey's agar. Isolates were examined from these colonies for various morphological characteristics, e.g. scale, shape, elevation, edge, inner surface and Gram stained smears were prepared. Cowan and Steel's

Manual for the Identification of bacteria was used to add the obtained isolates to several biochemical assays for species identification [23]. The Gram stain was applied to 18-24 hour cultures after the Hucker treatment [24]. A loop of an overnight society was air-dried and heat glued on a glass slide. The extra discoloration was rinsed away with a moderate stream of water. Iodine (0.4% w/v) was applied in grams and allowed to stand for 30 seconds before rinsing. The stain was rinsed with ethanol (95% v/v) before being stained for one minute with the secondary stain, safranin (0.4% v/v). It was then cleansed with water for 5 seconds. The bacterium showed pink under the microscope if it was Gram-negative.; however it looked purple if the cell was Gram-positive. After examining morphological and biochemical tests using standard clinical laboratory methods, the pure cultures of the selected bacterial isolates were identified [25].

**Histological and biochemical composition analysis**

Portions of infected organs (gills) were fixed in 10% formalin solution. Fixed samples were dehydrated and embedded in paraffin wax. The sections were then cut with a microtome at a thickness of 5-6 m, mounted on glass slides, and stained with several stains such as haematoxylin and eosin (H & E), Malory's stain, and periodic acid Schiff (PAS). Determination of carbohydrate and lipids were quantified according to the previous study [26], while Lowry's technique estimated the protein amount [27].

**Functionalization of MWNTs based filter and the filter design**

The industrial multiwalled carbon nanotubes (MWNTs), Taunit-M, were used as the filter in this study. This material is a loose black powder made up of many micrometers of granular MWNT agglomerates. Refluxing MWNTs in a mixture of concentrated $H_2SO_4$ and $HNO_3$ (3:1) introduced the functional groups.

The resulting mixture was then refluxed overnight at 100°C with constant stirring. After that, the MWNT acid combination was washed with deionized water until it reached pH 7, then dried at 100°C. To directly assess the efficacy of the purification and oxidation processes, the functionalized MWNTs (F-MWNTs) were weighted and studied using a scanning electron microscope equipped with energy dispersive X-ray spectroscopy. The F-MWNTs filter system was prepared as previously reported, [16], Fig 1. The efficiency of removal (R) expressed as:

$$R(\%) = \frac{C_0 - C}{C_0} \times 100$$

where the levels of the heavy metals in the aqueous solution are $C_O$ and C, in the initial state and after filtration in (mg/l).

## 3. Results

**Surface analysis of the MWNT filters**

The structure of tubes with small diameters is shown in Fig. 2a. The physical properties of F-MWNTs are shown in Table 1. The table showed small inner and outer diameters followed by high surface area. The specific surface area of F-MWNT is a significant factor that improves their filtration performance.EDS analysis determined the filter's composition, which is primarily carbon with a small increase in oxygen content due to the functionalization process, Fig. 2b. In comparison with pristine MWNTs, F-MWNTs show new peaks, [16], as shown by FTIR, Fig. 3. The very strong interactions between heavy metal ions and the hydrophilic surface of the functional groups are thought to be responsible for the removal of heavy metals by F-MWNTs [19]. C-O,-COOH, and -OH are the oxygenated function groups at 1115 $cm^{-1}$, 1630 $cm^{-1}$, and 3415 $cm^{-1}$, respectively, Fig. 3.

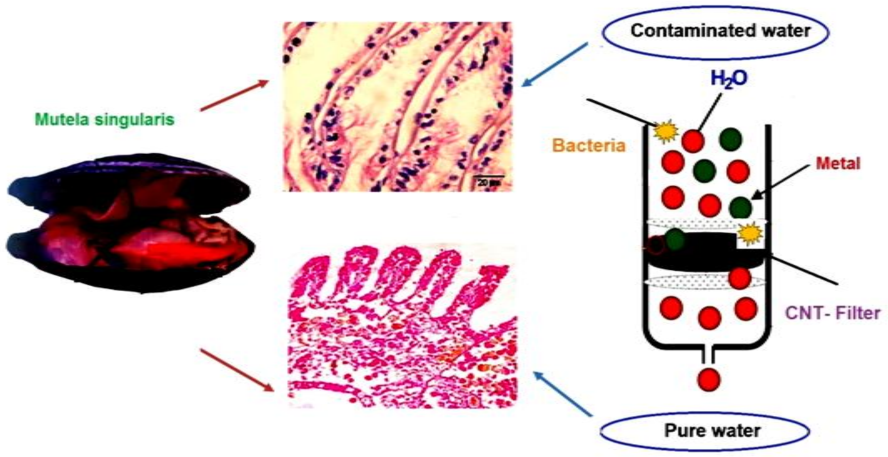


**Fig. 1** Schematic diagram for the design of MWNTs filter and the tissue enhancement of M. singularis

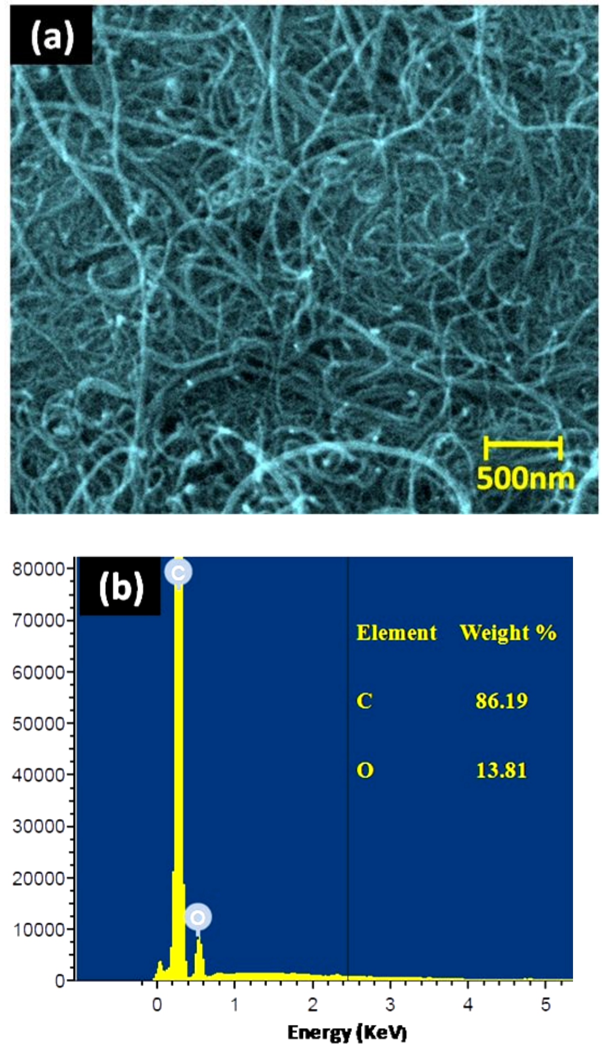


**Fig. 2**: SEM image of F-MWNT (a) and the EDS analysis showing the element content (b).

**Water analysis by bivalve *Mutela singularis***

Water samples from the investigated site (Abu Hummus, Egypt) were analyzed to detect the concentration of trace metals (Fe, Ni, Pb, Cd, Co, Cr, Zn, and Cu) after and before the living *M. singularis* which work as a heavy metal indicator.

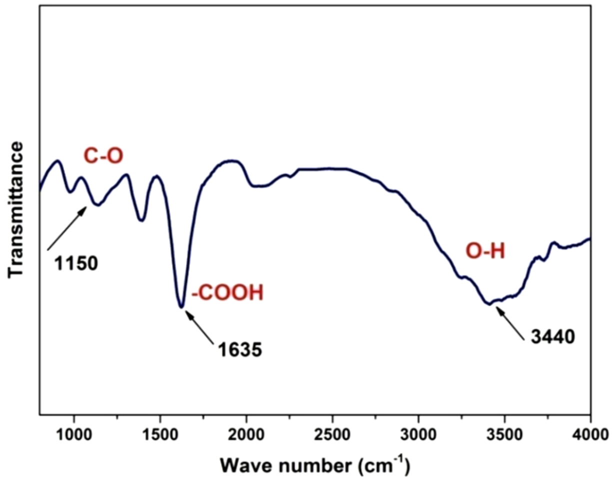


**Fig. 3:** The FTIR spectra show the groups of oxygen attached to F-MWNTs.

The order of accumulation of heavy metals in the water layer after filter was arranged as Fe >Zn > Cu >Cr> Co > Ni >Cd>Pb, as shown in Fig.4. A significant decrease in the metal concentration inside the water samples due to the absorption of these metals by the freshwater filter (*M. singularis*), these results are correlated with the pervious study [3]. These results show that the freshwater bivalve has the affinity to absorb the heavy metal species, which affect the structure of these types of Mollusks.

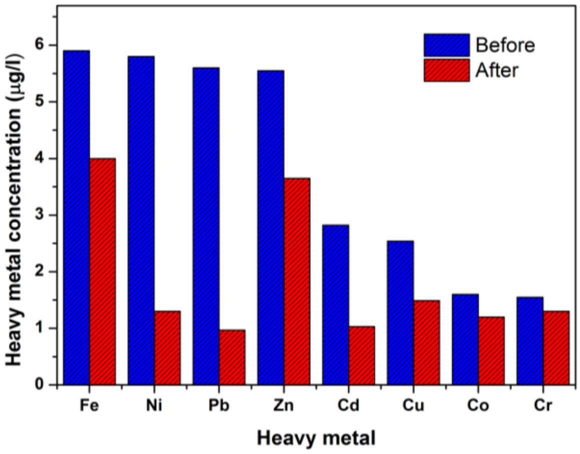


**Fig. 4:** The heavy metals concentrations in µg/L that collected from River samples in Abu Hummus before and after living of bivalve *M. singularis.*

**Table 1**: Physical characteristics of F-MWNT used in the current study

| **Physical parameter** | Outer diameter, nm | Internal diameter, nm | Tube length, µm | Specific surface area, $m^2/g$ |
|---|---|---|---|---|
| **Value** | 25 ± 2 | 7 ± 0.5 | 3 ± 0.2 | 212 ± 0.5 |

**The influence of pH and the filtration mechanism**

Physical adsorption, chemical interaction, and electrostatic attraction which rely on the pH solution are the most dominant mechanisms recorded in the adsorption literature [28]. The surface charge of CNTs is likely to become more negative due to the accumulation of more hydroxide ions as the pH of the solution increase [16], which increasing the ion exchange mechanism. On the MWNT surface, the heavy metal species are attached and the $H^+$ is extracted from the surface, decreasing the solution pH. Filtration data at pH of 3 (low pH) to 10 (high pH) and initial heavy metal concentrations of 5 µg/L and 0.2g F-MWNT filter mass are examined in the present investigation. From Fig. 4, the removal efficiency (*R*) of the metals, Co, Cd, Cr, Fe, Cu, Ni, Zn, and, Pb depends on the pH value. The graph shows that the high removal efficiency for the metals Co, Cu, Fe, Pb, and Zn was observed for high pH, but improved removal was noted for the low pH ions, Cd, Cr, and Ni. For copper, *R* is around 95% at pH=3, whereas at pH value of 10, *R* enhanced to reach 99.9%. Because of electrostatic interactions between the cation ions and negative surface charge ions, the adsorption of the cations Cd, Co, Cr, Cu, Fe, Ni, Pb, and Zn is higher at a pH value that elevates the point of zero charge ($pH_{PZC}$). On the contrary, due to neutralization of surface charge, the adsorption of these cations decreases at low pH [29]. F- MWNTs have been reported to have a lower $pH_{PZC}$ value than with raw tubes [30]. Two mechanisms describe the adsorption process of the divalent heavy metal ions on F-MWNTs as follows:

$$MWNT–OH^{2+}+M^{2+}\leftrightarrow[MWNT–OHM^{2+}]^{2+}+H^{+} \quad (1)$$
$$MWNT–O^{-}+M(OH)n^{2-n}\leftrightarrow[MWNT–O–M(OH)n^{2-n}]^{1-n} \quad (2)$$

Heavy metal ions are adsorbed at the surface due to competition between $M^{+2}$ and $H^+$ ions in the solution. However, $M(OH)_n^{2-n}$ is the dominant divalent metal ion species in the solution at pH greater than the $pH_{PZC}$.

It's worth noting that precipitation can happen at a higher pH, which has an impact on heavy metal ion elimination. For example the removal of lead is 96% at pH = 3. This is because the predominant lead species at low pH is $Pb^{2+}$ in a rivalry with $H^+$ ions to occupy the adsorption site. $Pb(OH)_2^0$and $Pb(OH)^+$, on the other hand, are the most common lead species at pH 10 and the removal reaches 99.6%, Fig.5.

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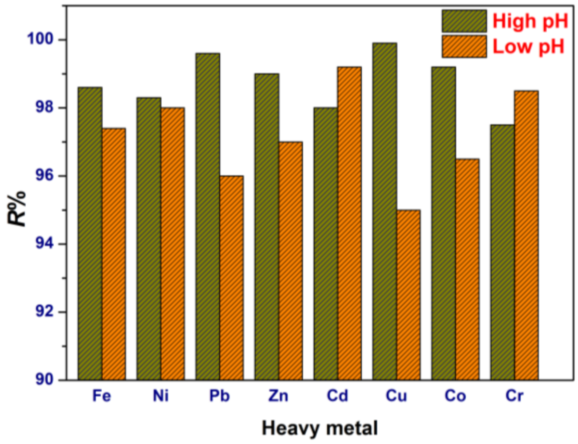


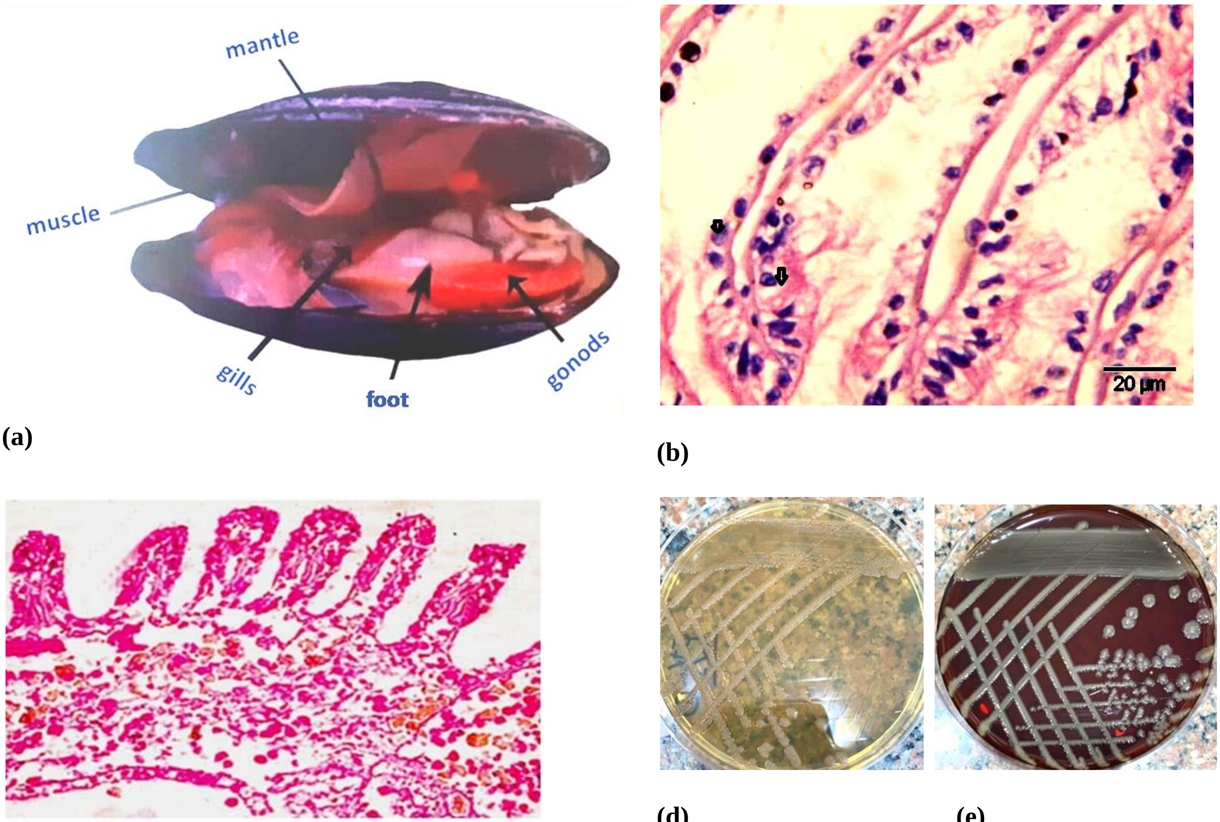

**Fig. 5**: The efficiency of MWNT-based filters for heavy metal of concentration 5 µg/L at different pH values

**Bacteria removal by MWNTs**

Apart from heavy metal removal, F-MWNTs exhibit strong antibacterial activity against gram-positive and gram-negative bacteria [18]. The defined bacteria (*S.aureus* & *V. alginolyticus*), Table 2, were isolated after and before using the F-MWNT filter from the water sample of *M. singularis*. It is clearly demonstrated that these bacteria have been completely eliminated after treatment that improves the aquatic conditions for the *M. singularis*. The membrane of these pathogenic bacteria is interrelated with F-MWNT, causing structural damage and cell death, and the mechanism for this association has been extensively dissected [17].

**Table 2**: Shows the levels of pathogenic bacteria in Gills and water samples before and after F-MWNT filters treatment.

| Type of bacteria | Before MWNT filtration | | After MWNT filtration |
|---|---|---|---|
| | Gills sample (cfu/ml) | Water sample (cfu/ml) | Percentage of bacteria In Gills and water samples |
| *S. aureus* | $3.52 \times 10^6$ | $5.8 \times 10^5$ | %0 |
| *V. alginolyticus* | $1.21 \times 10^6$ | $2.52 \times 10^6$ | %0 |




(c)

**Fig. 6**: A photograph shows the morphological structure of *M. singularis* (a). Photomicrograph showing gill lamella increased nuclear size, granular cytoplasm and nuclei, reduced inter-lamellar space, swollen gill lamellae and lamellar fusion, in the samples that lived in contaminated water (b). The samples in the purified conditions sh owing normal architecture of gill, lamellae, respiratory epithelium, cilia, nuclei and inter-lamellar (c). Disk showing representative isolates bacteria *S. aureus* (d), *V. alginolyticus* (e).

**Effect of F-MWNT filters on the aquatic conditions and the bivalve biochemical properties**

The *M. singularis* structure is showed in Fig.6a. Histological examination of gills of *M. singularis* bivalve using the optical microscope was presented in Fig.6 (b, c). Figure 6b shows the abnormal structure of gills due to presence of the heavy metals in surrounding water sample without treatment. After the water treatment by F-MWNT filters, it was observed a normal structure of gills that have been allowed to live in the purified aquatic media, Fig.6c. The representative isolates bacteria (*S.aureus*, V.*alginolyticus*) are shown in Fig.6 (d, e).

## 4. Discussion

Mollusks (e.g. *M. singularis*) are common species in freshwater ecosystems that are globally important, both ecologically and commercially [31]. As they serve as filter feeders, many pathogenic and toxigenic microorganisms can be concentrated in the Mollusks. The current study's histological examination revealed that the infection on the gills could lead to significant disease conditions. The infection of the Gills may interfere with the respiratory function of the investigated Mollusks. Mollusk bacterial infections have been widely observed [32].

**Table 3**: The mean content of Carbohydrate, Lipid, and Protein of *M. singular* before and after water treatment by F-MWNT filters.

| Tissue content of *M. singularis* (mg/g) | Carbohydrates Mean ± SD | Lipid Mean ± SD | Protein Mean ± SD |
|---|---|---|---|
| After F-MWNT | 16.13 ± 2.01 | 14.11 ± 2.31 | 134± 13.8 |
| Before F-MWNT | 10.53 ± 1.81 | 11.67 ± 1.91 | 94.42 ± 12.62 |

Because many freshwater bivalves amass and harbor a large commensally bacterial fauna, pathogenic bacteria are difficult to diagnose. Bacterial infections often have diverse effects on mollusks depending on their life stage. Soft parts of bivalve *M. singularis* were analyzed for showing the amount of carbohydrate, lipid, and protein in the samples lived in water before and after being treated with F-MWNTs, results indicated a significant increase in the value of these parameters in the samples lived in the treated aquatic media by F-MWNTs filters, Table 3.

Table 3 shows the enhancement in the tissue content of *M. singularis* after living in the treated water. It is worth seeing that the carbohydrates increased by about 60%, however, the lipids show a smooth increase after treatment. The protein content after enhancing the aquatic conditions gets higher by 45%, Table 3. The carbohydrate reserves can usually be used rapidly in aquatic species under unfavorable circumstances and the wide variation found in the tissues suggests that the mineralizable amount. In response to fluctuations in the nutritional state of the animal, carbohydrate reserves can fluctuate widely and quickly. In living tissues, protein is the most essential constituent with major metabolic and structural importance. In energy metabolism, cellular and subcellular membranes, lipids play a prime role. Table 3 shows an improvement in the number of carbohydrates, lipids, and proteins after the heavy metals and bacteria removal of from aquatic media using F-MWNT filters. These findings were consistent with the analysis of the depletion of protein content in *Macrobrachium kinesis* tissues subjected to different levels of the stress of tributyltin chloride on the protein metabolism [32]. It was concluded that there is a substantial decrease in the profiles of carbohydrates, proteins, and lipids in the digestive gland and gill after exposure to a certain cadmium concentration [33].

## 5. Conclusion

F-MWNT filters were employed to enhance the aquatic condition for the freshwater bivalves by removing the heavy metals and bacteria from River Nile. Prior to adopting F-MWNT filtration, our analysis generally captured the maximum amounts of heavy metals in the water sample. The filtration experiments indicated that the pH of the water samples is essential to control the removal efficiency of the metal species. As demonstrated by SEM, EDS, and FTIR, the functionalization process creates oxygen groups, reduces the tube diameter of the CNT-based filter, and increases its surface area. It was revealed that the metals Co, Cu, Fe, Pb, Ni and, Zn had high removal efficiency at high pH. However enhanced removal was noted for the ions, Cd, and, Cr at low pH with removal efficiency in the range of 95% to100%. *M. singularis* gills were examined histologically and the structure of the gill, lamellae and respiratory epithelium were improved. *M. Singularis* that lived in the treated water had high protein, lipid, and, carbohydrate ratios, indicating that the F-MWNTs are promising filters in aquaculture, irrigation, and mechanized farming. In the future, alternative applications of freshwater mussel rearing as an integrated development, bio-indication projects and the use of freshwater mussel meal as a possible source of fish feed should be studied in detail.

**Acknowledgement**

Thanks to the Physics department, Faculty of Science, Damanhour University, Egypt.

**References**

1. Arputha Bibiana, Manikandan M.S., Selvamani, P. and Latha S. (2014), Assesment of antimicrobial potency of Mactra Violaceae crude and protein isolates. Asian Journal of Microbiology, Biotechnology and Environmental Sciences 16(1):59-62
2. Rodriguez-Mozaz S., Chamorro S., Marti E., Huerta B., Gros M., Sànchez-Melsió A., Borrego C.M., Barceló D. and Balcázar J.L. (2015), Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Res. 1;69:234-242.
3. Casas S. and Bacher C. (2006), Modeling trace metal (Hg and Pb) bioaccumulation in the Mediterranean mussel, Mytilus galloprovincialis applied to environmental monitoring. Journal of Sea Research, 56,168-181.
4. Zilber-Rosenberg I. and Rosenberg E. (2008), Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution, FEMS Microbiol. Rev., 32 723-735.
5. Paillard C. and Roux F. L. , Borrego J. J. (2004), Bacterial disease in marine bivalves, a review of

recent studies: Trends and evolution. Aquatic Living Resources. 17:477-498.
6. Rallapalli S., Verghese S. and Verma R. S. (2008), Validation of multiplex PCR strategy for simultaneous detection and identification of methicillin resistant Staphylococcus aureus. Indian J. Medical Microbiol., 26(4), 361-364.
7. Lutz Carla, Erken Martina, Noorian Parisa, Sun Shuyang and McDougald Diane. (2013), Environmental reservoirs and mechanisms of persistence of Vibrio cholerae. Frontiers in Microbiology 4, 375-380.
8. El-Shenawy N. S. (2004), Heavy metal and microbial depuration of the clam Ruditapes decussatus and its effect on bivalve behavior and physiology. Environmental Toxicology, 19(2), 143-153.
9. Abdel Moneim A.M., Al-Kahtani M. A. and Dlmenshawy O. M. (2012), Histopathological biomarkers in gills and liver of Oreochromis niloticus from polluted wetland environments, Saudi Arabia. Chemosphere, 88(8) 1028-1035.
10. Forstner, U. and Wittmann, G. T. W. (1983), Metal Pollution in the Aquatic Environment. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo.
11. Bekhiet (2015), Determination of Heavy Metals in Fish Tissues and Water from White Nile Khartoum City, Sudan. J of Environ Protect and Sustainable Development, 1(3) 178-181.
12. Ayodele J. T. and Abu Bakar M.B. (2000), Trace metal levels in river wudil, Kano Nigeria Trop. Journal of Environmental Resources, 3, 230-237.
13. Palanivelu V., Vijayavel K., Balasubramanian S. and Balasubramanian M. P. (2005), Influence of insecticidal derivative (Cartap hydrochloride) from the marine olycheate on certain enzyme systems of the fresh water fish Oreochromis mossambicus. Journal of Environmental Biology 26(2), 191-195.
14. Macedonio F., Drioli E., Gusev A. A., Bardow A., Semiat R. and Kurihara M. (2012), Efficient technologies for worldwide clean water supply. Chemical Engineering and Processing, 51, 2–17.
15. Liu X., Wang M., Zhang S. and Pan B. (2013), Application potential of carbon nanotubes in water treatment: A review. Journal of Environmental Sciences, 25(7), 1263-1280.
16. Elsehly E.M., Chechenin N.G., Makunin A.V., Motaweh H.A. and Leksina E.G. (2017), Functionalized carbon nanotubes based filters for chromium removal from aqueous solutions. Water Science and Technology, 75(7) 1564-71.
17. Elsehly E.M., Chechenin N.G., Makunin A.V., Motaweh H.A. and Shemukhin A.A. (2018), Enhancement of CNT-based filters efficiency by ion beam irradiation. Radiation Physics and Chemistry, 146, 19-25.
18. Abo-Neima S., Motaweh H. and Elsehly E.M., (2020), Antimicrobial activity of functionalized carbon nanotubes against pathogenic microorganisms. IET Nanobiotechnology, 14 (6), 457–464.
19. Elsehly E. M.; Chechenin N.G.; Makunin A.V.; Pankratov D. A. and Motaweh, H.A. (2018), Ozone functionalized CNT-based filters for high removal efficiency of benzene from aqueous solutions. Journal of Water Process Engineering 25, 81-87.
20. Xu J., Cao Z., Zhang Y., Yuan Z., Lou Z., Xu X. and Wang X. (2018), A review of functionalized carbon nanotubes and graphene for heavy metal adsorption from water: Preparation, application, and mechanism. Chemosphere, 195, 51-64.
21. Elsehly E. M. (2020) Enhanced removal of Ni (II) from aqueous solutions by effective acid functionalization of carbon nanotube based filters. *The Egyptian Journal of Chemistry*, 63(10), 3861-3871.
22. Elsehly E. M., Chechenin N.G., Makunin A.V. and Motaweh H.A., (2016) Morphological and structural modifications of multiwalled carbon nanotubes by electron beam irradiation, Material research express 3, 105013. 10.1088/2053-1591/3/10/105013
23. Krieg N.R. and Holt J. G. (2001), Bergey's Manual for Systematic Bacteriology. Second Edition. Williams and Wilkins Pub-lishers, Baltimore.
24. Collins C.H. and Lyne P. M. (1985), Microbiological Methods.5th edition .Ed.C.H.Collins and P.M.Lyne. Butterworth, London.
25. Murray P.R., Baron E.J., Jorgensen J.H., Landry M.L. and Pfaller M.A. (2007), Manual of Clinical Microbiology, 9th Ed., ASM Press, Washington, D.C.
26. Allain C. C., Poon L. S. and Chan R.W. (1974), Enzymatic determination of total serum cholesterol. Clin. Chem., 20, 470-475.

27. Lowry O.H., Rosebrough N. J., Farr A. L. and Randall R.J. (1951), Protein measurement with foil phenol reagent, Journal of Biological Chemistry. 195, 265-275.
28. Fu F. and Wang Q. (2011), Removal of heavy metal ions from wastewaters: A review. J. Environ. Manage. 92, 407-418.
29. Tang W.W., Zeng G.M., Gong J.L., Liu Y., Wang X.Y., Liu Y.Y. Liu Z.F., Chen L., Zhang X.R. and Tu D.Z. (2012), Simultaneous adsorption of atrazine and Cu (II) from wastewater by magnetic multi-walled carbon nanotube, Chem. Eng. J. 211, 470-478.
30. Mustafa S. A. (2020), Histopathology and heavy metal bioaccumulation in some tissues of Luciobarbus xanthopterus collected from Tigris River of Baghdad, Iraq, The Egyptian Journal of Aquatic Research, 46(2), 123-129.
31. Amer M. S. and Ibrahim H. A. H. (2019), Chitosan from marine-derived Penicillum spinulosum MH2 cell wall with special emphasis on its antimicrobial and antifouling properties. The Egyptian Journal of Aquatic Research, 45(4), 359-365.
32. Kharat P.S., Laxmi B., Ghoble K. B., Shejule R., Kale S. and Ghoble BC. (2009), Impact of TBTCl on Total protein content in freshwater prawn, Macro brachium kistnensis., Khartoum city sudan, Middle –East Journal of Scientific Research. 4(3)180-184
33. Senthamilselvi P., Sukumaran M. and Muthukumaravel K. (2017), Toxicity of Cadmium on the biochemical composition and histology of Estuarine clam Meretrixcasta. International Journal of current research, 9 (11), 60271-60275.