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Impact of discharged textile dyes on environmental water bodies: A physicochemical, Eco-toxicological and microbiological assessment

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Abstract

Discharged textile wastewater without any treatment may have negative impact on the physio-chemical, microbiological features of domestic wastewater. Therefore, the aim of this study was to determine the physical-chemical, heavy-metals, toxicity, and microbiological characteristics of textile wastewater collected from five textile factories and surface water collected from River Nile, Ismailia canal and El-Rahawy drain water. The obtained results of physical-chemical parameters in textile wastewater influents showed that, pH ranged between 7.1 and 12.2. However, TDS ranged from 2348 to 7049.8 mg/L, TSS varied from 83.6 to 363 mg/L. Moreover, COD and BOD in influents of textile wastewater reached 319.7 and 902.7 mg/O_2L and 108.2 to 367.5 mg/O_2L, respectively. On the other hand, COD, BOD and oil in El-Rahawy drain water reached to 189.8, 77.8, and 9.3 mg/L, respectively. Bacterial indicator, some selected pathogenic bacteria and dye degrading bacteria (DDB) were detected in the majority of the collected samples. The highest values of total coliform (TC), fecal coliform (FC) and fecal streptococci (FS) in textile wastewater were reached6.05×10^5, 5.98×10^5 and 5.85×10^5 MPN/100 mL, respectively. The tested heavy metals in both textile wastewater and environmental water samples were within the permissible limits of law 44/2000. All textile wastewater and environmental water samples were toxic except samples collected from Jeans Care Factory and River Nile water. The textile wastewater was not complying with the environmental legislation (Law 48/1982 Ministerial Decree 92/2013). Efficient treatment process for textile wastewater before mixing with municipal wastewater is highly needed.

KeyWords: Textile wastewater, Physico-chemical, Bacterial pathogens, Dye degrading bacteria, Heavy metal, Toxicity

1. Introduction

Over 10,000 different dyes and pigments are utilized in the textile industries, and more than 7 x 10^5 tonnes are produced globally each year [1]. The use of dyestuff is rapidly expanding because of the textile industry's explosive growth. Due to their wide range of color tones, high wet intensity profiles, simplicity of use, brilliant colors, and low energy consumption, synthetic dyes are frequently used in the clothing industry[2]. Clothing, paper, leather, fruit, cosmetics, and the pharmaceutical industries all frequently utilize azo dyes [3]. Azo dyes are the most prevalent class of synthetic dyes released into the atmosphere, and represented by 60- 70% of all dyes used globally [4, 5]. According to recent estimates, untreated synthetic dyes are discharged into water bodies at a rate of more than 280,000 tonnes annually worldwide. Synthetic dyes are not only unpleasant, but they also commonly restrict light penetration into the water, which has an impact on the ability of aquatic organisms to perform photosynthesis due to the presence of aromatics, metals, chlorides, etc. [6, 7].

Wastewater from textile industries contains azo dye residue and its metabolites, which are known to be detrimental to humans and the environment. Wastewater with azo dye residue has colors and has an unappealing aesthetic [8]. Aside from that, azo dyes and their metabolites harm aquatic ecosystems by being acidic, mutagenic, teratogenic, and carcinogenic to living organism's [9, 10]. Additionally, the marine ecosystem suffers when azo

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dyes with highly colored effluent are disposed of in large quantities. Diarrhoea, vomiting, exhaustion, stomach discomfort, and disorientation are the most typical signs of dye exposure in humans [11]. Presence of textile dyes in wastewater is considered one of the major matters in management textile wastewater using conventional treatments. This is partially due to presence of different types of fiber staining such as cellulose, protein, polyester, nylon and polypropylene synthetic fibers [12]. In addition to this, textile sludge displays issues related to excess quantities and undesired structure, often with elevated micronutrients, organic matter loads, heavy metal cations and microbial pathogens [13].

Chemical analyses can only measure a small number of pollutants, and they are not necessarily consistent with wastewater's hazardous effects [14]. In wastewater treatment facilities, toxicity tests show that combined pollutants have negative biological impacts, which can be used to infer wastewater removal effectiveness indirectly [15]. The treatment of textile effluents is crucial for both reuse and environmental protection because they are undesired. To choose the most suitable treatment procedure, the physical, chemical, and microbiological aspects of textile wastewater must be considered [16]. It is strongly advised that the effluents of textile industries be well-treated by combined treatment processes before being disposed of into the nearby water bodies in order to reduce the pollution load and avoid adverse pollution effects. Additional measured physico-chemical parameters include temperature, color, pH, dissolved oxygen (DO), electrical conductivity (EC), biological oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), total alkalinity, total hardness, and sodium (Na) [17]. Therefore, the objective of the current study was to determine the physical-chemical, some heavy-metals, acute toxicity, and microbiological characteristics of textile wastewater effluents and some environmental water bodies in order to provide an overview of the most polluted sites and to identify the best treatment strategy.

2. Experimental

2.1. Sampling sites

Textile wastewater samples and environmental water samples were collected for studying the effect of pollution load on the environment. The textile wastewater samples were collocated from five factories namely Factories from 1 to 4 located at Greater Cairo, while factory 5 located at Menofia Governorate. Furthermore, environmental water samples were collected from surface water including Ismailia canal, River Nile, and El-Rahawy Drain.The samples were collected six times each two weeks for three months in 2020.

2.2. Sampling collection

All samples were collected and transmitted according to APHA (2017) [18]. For microbiological analysis, the samples were collected in sterile one-liter wide-mouth bottle in duplicates. While for physicochemical and heavy metal analyses, the water samples were collected in cleaned polyethylene bottles. The samples for heavy metals analysis were acidified to less than pH (2) using conc. HCl and $HNO3$. The collected samples were transmitted in an icebox to the laboratories within 4 h.

2.3. Microbiological examination

2.3.1.Detection of bacterial indicator

Total bacterial counts (TBC), total coliform (TC), fecal coliform (FC) and fecal streptococci were determined according to APHA (2017). TBC were determined by pour plate method using standard plate count agar and the results were expressed as CFU/mL, while TC, FC and FS were determined using Most Probable Number (MPN) method and the results were expressed as MPN-index/100mL.

2.3.2. Detection of some pathogenic microorganisms

E. coli, *Pseudomonas aeruginosa, Salmonella* spp., *Staphylococcus aureus* and *Listeria monocytogenes* were determined using spread plate method according to APHA (2017). Samples or their appropriate dilutions were inoculated on selective-differential growth media that imported from HiMedia, India. *E. coli* was detected on Rapid HiColiform agar. The blue colonies were typical *E. coli*. While, *Pseudomonas aeruginosa* were detected on Hifluoro Pseudomonas agar. *P. aeruginosa* colonies produced a visible fluorescence under long-wave UV light. Furthermore, *Salmonella* spp were detected onto the Hicrome Improved Salmonella agar. The typical colonies of *Salmonella* spp. were light pink color. Additionally, *Staphylococcus aureus* was detected on the Hicrome Aureus agar. *Staphylococcus aureus* colonies were brown-black, with a clear zone around the colony. Moreover, *Listeria monocytogenes* was detected on Hicrom Listeria selective agar. The color of typical *Listeria monocytogenes* colonies was bluish-green.

2.3.3. Isolation and screening of dye degrading bacteria

All types of the collected samples were used for the isolation of dye degrading bacterial strains. A total of ten dyes in addition to mixed dye from the ten dyes were used. The three reactive dyes, two direct dyes, and fast green, Methyl red (MR), crystal violet (CV), malachite green (MG), methylene blue (MB), and indigo dyes were used. All details about Isolation and phenotypic using BIOLOG and genotypic using

16S rRNA identifications of the dye degrading isolates were mentioned in [19].

2.4. Acute toxicity bioassay

Toxicity test was carried out using Microtox analyzer 500 instruments for all samples types. 10 µL from tested water samples or from its dilution were added to 0.5 mL of Microtox diluent. The Microtox analyzer Model 500 is fully automated and temperature controlled and needs no daily adjustment or calibration. A marine luminescent bacterium *Vibrio fischeri* (earlier referred as *Photobacterium phosphoreum*) has been widely used for acute toxicity estimation with several commercial tests. The toxicity bioassay is based on the detection of light output changes through the light production (which directly relative to the metabolic activity of the bacterial population) and any inhibition of enzymatic activity causes a corresponding decrease bioluminescence. EC_{50} and Toxicity levels were calculated according to [20].

2.5. Physicochemical analyses

Physicochemical parameters including; color, turbidity, pH, total suspended solids (TSS), total dissolved solid (TDS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total kjeldahl nitrogen (TKN), total phosphorous (TP), oil and grease, settleable solids, nitrite (NO_2), nitrate (NO_3) and ammonia (NH_3) were determined in all collected samples according to APHA (2017).

2.6. Heavy metals determination

The concentrations of heavy metals in all samples were determined after digestion, according to APHA (2017). Samples are dried in an oven at 105ºC for 2 days and subsequently ground in an agate mortar. Furthermore, to normalize the variations in grain size distributions, the dried sediment samples are sieved to 0.2 mm using stainless steel sieve for the analysis (APHA 2017). All heavy metals analyses were performed by an Atomic Absorption Spectrometer. The metal measurement precision was determined by analyzing (in triplicate) the metal concentration of all samples and for each series of measurements, an absorption calibration curve was constructed composed of a blank and three or more standards.

2.7. Statistical analysis

All experiments were conducted in triplicate and the data obtained were expressed as average ± standard deviation (SD) using Excel (Microsoft 2010).

3. Results and discussion

The physical, chemical, and microbiological characteristics of textile wastewater (collected fromfive Textile factories) were determined. Furthermore, environmental water samples were collected from the River Nile, Ismailia Canal, and El-Rahawy Drainfor studying the effect of pollution load on the environment.

3.1. Physico-chemical parameters for tested samples

The Average and ± standard deviation (STD) for some physico-chemical parameters for five textile factories and environmental samples were calculated and listed in **table (1)**.

The results revealed that pH of the textile industrial influents for the factories varied from 7.1 to 12.2, the differences attributed to the use of different dyes in various textile industries[21]. While the pH of the industrial effluent for five factories ranged from 7.7 to 8.4. TDS is the indicator for the total ionic components of wastewater, the high concentrations of TDS have a negative impact on agriculture and the aquatic organism [22]. The average value of TDS in influents of textile factories ranged from 2348 to 7049.8 mg/L while for effluent ranged from 1637.5 to 6170 mg/L.On the other hand, TSS represents particulates in waters and their detection is typically responsible for holding pollutants in the aquatic environments as well as a decrease in oxygen (O2) levels in water bodies [23]. The average value of TSS varied from 83.6 to 363 mg/L and from 55.3 to 254.7 mg/L in the influent and effluent of textile factories, respectively. COD and BOD are the two main properties that reveal the load of wastewater. Both parameters investigate the oxygen-demanding strength of the wastewater. The load of influent of textile factories ranged from 319.7 to 902.7 mg/O_2L for COD and from 108.2 to 367.5 mg/O_2L for BOD, while the load of effluent ranged from 226.2 to 894.7 mg/O_2L for COD and from 95 to 307 mg/O_2L for BOD. TKN, it is used for determination of organic nitrogen and ammonia. The results confirmed that the TKN value in the influent and effluent of textile factories varied from 5.3 to 25.1 mg/L and from 14.5 to 19.8 mg/L, respectively.

Total phosphorus (TP) evaluates the all forms of phosphorus that are found in water. The results demonstrated that TP of the industrial influents of the factories ranged from 9.8 to 20.08 mg/L while for effluents ranged from 9 to 34.2 mg/L. Oil and grease are a major challenge in the treatment of wastewater because if they weren't removed from wastewater before discharge can interfere with biological life in surface waters and lead to serious environmental problems. The results exhibited that the oil and grease in the influent and effluent of textile factories varied from 12.2 to 49.7 mg/L and from 8.7 to 28.4 mg/L, respectively.

Textile wastewater effluent of Factory (5) was treated by adding antibacterial agent in addition to sulfuric acid diluted. The final effluent after treatment was discharged in public industrial wastewater pipeline separated on domestic wastewater. Overall, the results indicated that the wastewater content from Factory (5) was heavily polluted with organic and inorganic chemicals before and after treatment. This could be due to the inefficiency of coagulation treatment in terms of flow rate and chemical doses. Moreover, textile wastewater effluent of factory (1) was treated using trickling filter and addition of coagulant polymer agent. The final effluent after treatment was discharged in public industrial wastewater pipeline separated of domestic wastewater. The obtained results showed that, factory (1) the treatment unit achieved about 50% removals of contaminants. In the case of factory (3), mixing the textiles with municipal wastewater resulted in the neutralization of wastewater and reduction of organic and consequently, the quality of textile effluent complying with the environmental legislation. On the contrary, in factory (2), the quality of textile effluent after mixing with municipal wastewater was not complying with the environmental legislation (Law 48/1982 Ministerial Degree 92/2013).The study also highlighted the negative impact of anthropogenic activities on surface water and the importance of addressing environmental issues. As shown in table (1), the characteristics of surface water from Nile and Ismailia canal showed normal values of raw water within environmental legislation, while analyses of El-Rahawy drainage water show the contamination of drainage with high amounts of organic pollutants, whereas, COD, BOD and oil which reached to 189.8, 77.8, and 9.3 mg/L, respectively. Law 44/2000 about the disposal of agricultural wastewater into urban wastewater affects Egypt. pH 6-9.5, COD 1100 mg/L, BOD 600 mg/L, TSS 800 mg/L, TKN 100 mg/L, oil, and grease 100 mg/L were the acceptable limits.

3.2. Classical bacterial indicator and some pathogenic bacteria

The Average and ± standard deviation (STD) for classical bacterial indicator including total bacterial counts (TBC), total coliforms (TC), fecal coliforms (FC), and fecal streptococci (FS)) and some pathogenic bacteria (*E. coli,Salmonella* spp., *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Staphylococcus aureus*) for five textile factories and environmental samples were calculated and listed in the table (2). The results showed the presence of high levels of TBC, TC, FC, and FS in both the textile industrial influents and effluents of the factories, where TBC at 37 and 22^{o}C of the industrial influents for five factories varied from 1.92×10^3 to 6.62×10^5 CFU/mL and from 1.54×10^3 to 69.23×10^5 CFU/mL, respectively. While for effluents TBC at 37 and 22^{o}C ranged from 9.07×10^3 to 1.88×10^8 CFU/mL and from 7.8×10^3 to 1.46×10^8 CFU/mL, respectively. TC, FC, and FS have all been used to measuring the disinfection efficiencies of wastewater [24]. The average value of TC in influents of textile factories ranging from 6.5x10 to 2.27×10^4 MPN/100mL, in addition to, TC in effluent samples ranging from $2.1\text{x}10^3$ to 6.05×10^5MPN/100 mL.

On the other hand, the average value of FC varied from 4.7x10 to 1.93×10^4 MPN/100 mL and from $1.5\text{x}10^3$ to 5.98×10^5 MPN/100mL in the influent and effluent of textile factories, respectively. Furthermore, the FS of influents of textile factories ranged from 1.0 to 4.12×10^3 MPN/100 mL, while the FS of effluent ranged from 2.33 to 5.85×10^5MPN/100 mL. Pathogenic bacteria in wastewater cause human diseases [25]. Waterborne diseases are greatly influenced by the bacteriological characteristics of textile wastewater, which serve as reliable contamination markers. The biogeochemical cycle and energy transfer in the aquatic environment depend on microorganisms [26]. In current study, *E. coli*, *Salmonella spp.*, *P. aeruginosa*, *L. monocytogenes*, and *S. aureus* were measured. The results confirmed that the average E. coli values in the influent and effluent of textile factories varied from 1.67 to 2×103 CFU/100mL and from 1.6 to 1.22×104 CFU/100mL, respectively. The average density of *Salmonella* spp. in the wastewater influent of textile factories ranged from $3.4\text{x}10^2$ to 1.65×10^3 CFU/100mL. While the density of *Salmonella* spp. in the textile wastewater effluent ranged and from $8.0\text{x}10^2$ to 7.1×10^3 CFU/100 mL. The density of *P. aeruginosa* in the industrial wastewater effluent ranged 4.75×10^2 to 8.92×10^3 CFU/100mL. Furthermore, *L. monocytogenes*, and *S. aureus* were found in most collected samples and varied from $4.2\text{x}10^2$ to 1.70×10^3 CFU/100mL for *L. monocytogenes* and from 2.6x10 to 9.5×10^2 CFU/100 mL for *S. aureus* in influent samples, while from 1.37×10^2 to3×10^3 CFU/mL for *L. monocytogenes* and from 8.5 to 8.7×10^3 CFU/mL for *S. aureus* in effluent samples. In conclusion, the results obtained illustrated that the classical and pathogenic bacterial indicator were detected in most collected samples from five industrial factories and not complying with environmental Law 48/1982 Ministerial Decree 92/2013.

In the present study, mixing the effluent of textile wastewater with municipal wastewater leads to change in bacteriological community structures in surface water. In many industries and factories do not have sufficient means of disposing of their waste thus, the waste quickly finds its way into nearby streams that gradually trickle into surface water and drains which affected on the quality of surface water as shown in table (2), classical and pathogenic bacterial indicators were detected in most collected of samples from Nile, Ismailia canal, and El-Rahawy drainage.

3.3. Dyes degrading bacteria for tested samples

All types of the collected samples were used for the isolation of dye degrading bacterial strains. A total of ten dyes (Reactive blue (RB), Congo red (CR), Methyl red (MR), Reactive red (RR), Direct red (DR), Direct blue (DB), Crystal violet (CV), Fast green (FG), Malachite green (MG), Methylene blue (MB)) in addition to mixed dye from the ten dyes were used. The Average and ± standard deviation (STD) for dyes degrading bacteria for five textile factories and environmental samples were calculated and listed in the table (3). The results showed that the average counts of dyes degrading species of influent of textile factories ranged from 1.08×10^3 to 3.47×10^3CFU/mL for RB, from 3.42×10^2 to 2.83×10^3 CFU/mL for CR, from 4.22×10^2 to 2.77×10^3 CFU/mL for MR, from 5.83×10^2 to 3.52×10^3 CFU/mL for RR, from 4×10^2 to 3.83×10^3 CFU/mL for DR, from 1.04×10^2 to 3.13×10^3 CFU/mL for DB, from 2.0×10 to 7.25×10^3 CFU/mL for CV, from 7.5×10 to 1.48×10^4 CFU/mL for FG, from 5×10 to 3.6×10^3 CFU/mL for MG, from 2.72×10 to 3.57×10^3 CFU/mL for MB, and from 1.35×10^2 to 2.04×10^4 CFU/mL for Mixed dyes. Furthermore, the results illustrated that the average counts of dyes degrading species of effluent of textile factories ranged from 1.02×10^2 to 5.14×10^3 CFU/mL for RB, from 3.27×10^2 to 5.05×10^3 CFU/mL for CR, from 5.87×10^2 to 4.35×10^3 CFU/mL for MR, from 2.06×10^2 to 4.28×10^3 CFU/mL for RR, from $2.82\times10$to 4.73×10^3 CFU/mL for DR, from 8.33×10 to 3.3×10 CFU/mL for DB, from 1.2 to 1.97×10^3 CFU/mL for CV, from 1.84×10 to 3.2×10^3 CFU/mL for FG, from 7.25 to 3.5×10^3 CFU/mL for MG, from 4.07×10^2 to 3.77×10^3 CFU/mL for MB, and from 8 to 2.3×10^3 CFU/mL for Mixed dyes. Thus, we can conclude from the results that dying degrading bacteria have the ability to grow on all examined dyes even after treatment in case of factories number 1 and 5 or after mixing with municipal wastewater in case of factories 2, 3 and 4.

Also, from the results of environmental samples we can conclude that the biodegradable bacteria on all tested dyes were detected in most collected samples from Nile, Ismailia canal, and El-Rahawy drainage. Bacteria can breakdown and demineralize organic/inorganic contaminants, which is crucial for removing pollutants from textile wastewater [27]. According to [28], the high quantities of organic matter in wastewater encourage the quick growth of microbes that use oxygen and deplete the dissolved oxygen in the water, creating septic conditions or anoxia that is fatal to aquatic life.

3.4. Heavy metals of the tested samples

Heavy metals are widely used for the production of color pigments of textile dyes. High concentrations of metals in waste streams lead to a daily discharge of several kilograms of metals into receiving water bodies. Determination of heavy metal concentration in industry wastewater and natural water system has received increasing attention for monitoring environmental pollution because some metals are not biodegradable. The metals of the tested samples for five textile factories and environmental samples (River Nile, Ismailia Canal, El-Rahawy drain) were calculated and listed in the table (4). The results showed that Lead, Cadmium, Molybdenum, Mercury and Cobalt were not detected in all collected samples from factories and environmental samples. However, other metals such as Fe, Mn, Cu, Zn, As, Pb, Cr, and Ni were founded. The most contaminating metal was iron with a maximum concentration of 51.5 mg/L for Factories 1 and 4. Mn, Cu, Zn, As, Pb, Cr, and Ni were also present in some collected samples and ranging from 0.03 to0.45, <0.01 to 0.15, <0.01 to 3.33, <0.001 to 0.03, <0.001 to 0.35, <0.001 to 0.03, and <0.001 to 0.2, respectively.

Some heavy metals including; Iron (Fe), Manganese (Mn), Copper (Cu), Zinc (Zn), Arsenic (As), Lead (Pd), Total Chromium (Cr), Cobalt (Co), Cadmium (Cd), Nickel (Ni), Molybdenum (Mo) and Mercury (Hg) in textile wastewater samples were within the permissible limits of law 44/2000 [29]. The majority of the tested heavy metals were higher than the permissible limits in sludge samples. On the other hand, heavy metals were within the Egyptian Law for protection of water Law 48/1982 [30] in water samples collected from River Nile, Ismailia canals, El-Rahawy drain.

3.4. Toxicity of the tested samples

Toxicity is usually determined by the substance's ability to harm an organism and depends on the chemical properties of the compound and its concentration. It is also determined by the duration and frequency of exposure to toxins and the substance's relationship to the organism's life cycle. The Microtox toxicity test has been used for toxicity testing. The effects of toxicity are expressed as EC_{50}, which is an inversely proportional metric [31]. The values of Microtox® EC 50% concentrations and toxicity level of the tested samples for five textile factories and environmental samples (River Nile, Ismailia Canal, El-Rahawy drain) were calculated and listed in the table (5).The results illustrated that textile wastewater before and after treatment from factory (5) was toxic which suggests that the treatment process used in the factory was not effective in reducing the toxicity of the sample, while from factory (4) was nontoxic with $EC_{50}\%$ =249. On the other hand, Factory (1) sample was initially nontoxic, but after treatment, it became extremely toxic, this could be caused by complex colour molecules oxidising to smaller hazardous chemicals. After degrading, some of these dyes and chemicals get up in the effluents of the clothing industry and becoming hazardous before the finished product is ready [32]. This suggests that the treatment process used in the factory had a negative impact on the sample. The factory (2) garment wastewater findings indicate that the effluent was non-toxic (EC50% = 314) before combining with municipal wastewater. After combining with urban wastewater, though, the effluent was very toxic (EC_{50}% = 23). The clothes effluent's sludge was dangerous since it included a lot of color. These color and chemicals have an unsuitable appearance, and their hazardous effects can contaminate the atmosphere once they break down [33]. Similarly, factory (3) findings, it is observed that textile wastewater was very toxic before and after mixing with municipal wastewater. These results recommend that the mixing process used in the factories may have contributed to the toxicity of the samples.

Table (5): The values of Microtox® EC50% concentrations from textile wastewater collected from five textile factories and environmental samples

Sample source		EC 50% Conc.	Toxicity results
Factory 1	before treatment	218	Nontoxic
	after treatment	14	Extremely toxic
Factory 2	before mixing	314	Nontoxic
	after mixing	23	Very toxic
Factory 3	before mixing	29	Very toxic
	after mixing	31	Very toxic
Factory 4	before mixing	249	Nontoxic
Factory 5	before treatment	48	Toxic
	after treatment	41	Toxic
River Nile		200	Nontoxic
Ismailia Canal		72	Moderately toxic
El-Rahawy		43	Toxic

For environmental samples, River Nile was nontoxic (EC_{50}% = 200), suggesting that the water quality in the river is good. While Ismailia Canal was moderately toxic (EC_{50}% = 200), and El-Rahawy water was toxic (EC_{50}%= 43) which signifying that the water quality in the area is not suitable for aquatic life. Underlying soil, sediment, and surface water contamination is a serious issue on a global scale. The environment must be protected by the treatment of textile effluents so that the treated effluent can later be recycled for irrigation or used in the textile factory's activities [34].

The Microtox toxicity test is based on the relationship between the reduction of light produced by *Vibrio vischari* bacteria and the sample's toxicity. The bioluminescence reaction of these bacteria is linked to the electron transport system in their cellular respiration and indicates the cell's metabolic status.The results reported by the Microtox tests are presented as effective concentration (EC50). However, to standardize the results and the correlation with physicochemical parameters from the Pearson index, toxicity units (TU50) were calculated using the following formula: {TU=$\mathbf{100/EC50\,(15min)}$}.

The toxicity level determine according to (Modern Water Incorporation, 2016) (Table S1).

Table 5 shows the toxicity results and EC50% of all tested samples. Textile waste of factories 2 and 3 were very toxic. Some environmental samples in El-Rahawy and Ismailia canal were toxic while River Nile water was nontoxic.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Table (1): The Average and ± standard deviation (STD) for some physico-chemical parameters for five textile factories and environmental samples

Parameters	Unit	Average ± STD													
		Factory 1		Factory 2		Factory 3		Factory 4		Factory 5		River Nile	Ismailia canal	El-Rahawy drain	Law 48/1982 Ministerial Degree 92/2013
		Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent				
pH	--	9.9± 0.98	8.3± 0.17	7.1± 0.91	7.74± 1.3	7.9± 1.6	7.8± 1	9.2± 1.8	8.4± 1.1	12.2± 0.37	8.1± 1	7.5± 0.5	8± 0.2	7.6± 0.2	6-9
TSS	mg/l	258.5± 175.7	189.3± 233.3	36.3± 18.3	254.7± 340.8	83.6± 93.8	55.3± 26.3	271.5± 75.1	245.2± 33.2	194.8± 156.8	115.1± 76.4	12.3± 0.9	12.8± 3	90.2± 7.2	30
TDS	mg/l	5877.5± 6178.8	1637.5± 1512.8	3348.7± 2330.3	4593.3± 1352.6	6368.5± 10671.1	2217.3± 762.3	2348± 632.6	3282.5± 646.1	7049.8± 7089.8	6170± 2165.8	269.5 ±10.7	279.3± 7.1	1042.2± 260.6	800
Color	unit	708.2± 288.1	274.5± 214	1531.7± 645.1	1208.8± 527.4	278.5± 303.4	221.7± 97.8	1047± 254	1176.7± 192.1	570.5± 207.7	976.3± 314.1	2.9± 0.7	13± 1.4	29.5± 12	--
Turbidity	NTU	96.3± 24.8	56.6± 38.8	282.2± 120.3	158.5± 126.6	67.8± 26.7	75.9± 62.1	168.5± 77.1	125.2± 4.3	62.8± 39.3	75.8± 48.1	4.8± 1.6	2± 0.6	62.7± 22.6	--
COD	mg/l	902.7± 272.6	499.8± 108.3	553.2± 136.1	544.7± 220.1	319.7± 238.1	226.2± 112	537.5± 193.5	375.2± 268.9	719.5± 227.0	894.7± 247.3	13.7± 2.3	17± 4.2	189.8± 32.6	20
BOD	mg/l	367.5± 153.5	179.7± 32.9	216.5± 66.9	209.7± 105.4	108.2± 85.5	59.5± 43.7	128.8± 36.8	95± 61.4	237.5± 90.9	307± 116.1	6.5± 0.6	8.5± 1.3	77.8± 10	20
TKN	mg/l	18.5± 15.1	15.8± 14.6	5.3± 2.7	19.8± 9.1	11.9± 2.9	14.5± 3.3	25.1± 11.7	14.8± 7.1	16.2± 4.5	16.2± 9.2	5± 1.3	3.6± 1	23.9± 3.1	--
NH3	mg/l	7.84± 12.4	7.8± 10.5	0.78± 1.2	7.32± 9.8	1.7± 2.6	3.2± 1.1	10±8.2	1.1± 1.2	1.9± 1.4	0.96± 0.79	N.D	N.D	11.5± 5.4	--
NO2	mg/l	3.4±6.8	3±6	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	--
NO3	mg/l	7.4± 12.2	0.39± 0.46	13.4± 26.6	22.3± 30.8	22.8± 55.8	1.9± 4.6	5.1±2.6	2.5±0.6	2.8± 1.1	0.76± 0.43	0.4± 0.3	0.24± 0.08	0.1± 0.2	30
T.P	mg/l	14.7± 10.3	14± 11.1	11.4± 3.4	15.4± 6.9	9.8±9.7	9± 3.7	14.1±8.4	18.8± 3.8	20.08± 11	34.2± 29	2± 0.4	1.4± 0.2	10.5± 7.5	--
O.G	mg/l	49.7± 14.2	25.7± 5.9	21.1± 6.3	25.3± 9.5	12.2± 10.6	8.7± 8.6	13.7± 4.8	9.6± 1.5	16.4± 6.9	28.4± 20.3	N.D	N.D	9.3± 1.7	5

Table (2): The Average and ± standard deviation (STD) for some Classical bacterial indicators, and some pathogenic bacteria for five textile factories and environmental samples

Parameters		Unit	Average ± STD													
			Factory 1		Factory 2		Factory 3		Factory 4		Factory 5		River Nile	Ismailia canal	El-Rahawy drain	Law 48/1982 Ministerial Degree 92/2013
			Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent				
Classical bacterial																
Total bacterial counts	22oC	counts (CFU/ mL)	3.67E+04±32543.3	7.87E+03±9902.3	6.53E+03±8718.6	2.95E+06±2466311	3.38E+04±33434.5	4.04E+04±35652.2	1.54E+03±2196.2	1.46E+08±2.83E+08	9.23E+05±1322448	6.30E+05±816694.7	7.15E+03±14142.1	1.97E+04±18715.8	1.76E+08±1.39E+08	--
	37oC		4.98E+04±29929.4	9.07E+03±9994.1	8.25E+03±9547.1	2.64E+06±1888475	3.85E+04±38058.4	5.03E+04±51520	1.92E+03±2797.9	1.88E+08±8E+06	6.62E+05±942813.2	1.05E+06±141132	7.99E+03±15706.3	2.55E+04±26471.6	1.29E+08±1.34E+08	--
Total coliforms		MPN-index/100 mL	2.27E+04±27205.5	3.13E+03±4439.6	2.56E+03±4207.2	2.01E+05±278655.2	2.82E+03±3604.3	2.11E+03±3628.9	6.50E+01±95	6.05E+05±1020833	N.D	ND	1.90E+03±3640.3	4.38E+03±9647.7	9.10E+06±4248529	1000
Fecal coliforms			1.93E+04±24257.6	2.94E+03±4243.6	1.35E+03±1725.4	1.26E+05±188739.7	1.53E+03±1763.8	2.11E+03±3628.9	4.75E+01±65.8	5.98E+05±1025376	N.D	ND	2.18E+02±137.9	1.04E+02±97.7	1.10E+07±0	--
Fecal streptococci			4.12E+03±5436.7	7.60E+02±1548.4	3.67E+03±4954.3	4.07E+04±46392.5	1.78E+03±953.8	1.50E+02±116.4	1.89E+01±39.9	5.85E+05±1034133	1.00E+00±1.5	2.33E+00±3.6	2.62E+02±185.9	2.83E+02±362.3	1.08E+07±500000	--
pathogenic bacteria																
E. coli		(CFU/100 mL)	7.68E+02±820.3	1.06E+03±1657.5	1.85E+03±1323.2	2.28E+03±655.5	2.00E+03±1634.3	6.17E+03±9304.9	1.67E+00±4.1	1.22E+04±23970.5	1.54E+03±2511.7	N.D	1.11E+03±654.7	1.66E+03±895.8	2.80E+03±1042.4	--
Salmonella spp.			4.79E+02±901.4	8.08E+02±1199.3	1.65E+03±1698.1	2.63E+03±1721.2	1.21E+03±1010.6	7.11E+03±15156.1	N.D	1.94E+03±1256.2	3.42E+02±716.5	N.D	5.60E+02±518.7	1.11E+03±502.4	3.18E+03±1519.6	--
Pseudomonas aeruginosa			1.13E+03±880.4	1.24E+03±1716.5	1.89E+03±1452	3.02E+03±1629	1.48E+03±1541.1	8.92E+03±16239	N.D	1.57E+03±940.6	4.22E+02±969.4	4.75E+02±842.2	1.13E+03±508.2	1.76E+03±1603.8	3.35E+03±435.9	--
ListeriListeria monocytogenes			8.97E+02±1042.2	3.75E+02±750	1.70E+03±1402.5	3.00E+03±1597.5	1.37E+03±1072.3	2.98E+03±3966.3	N.D	2.89E+03±3453.6	5.83E+02±718.9	1.37E+02±324.7	8.38E+02±471.5	1.19E+03±974.5	2.93E+03±359.4	--
Staphylococcus aureus			5.56E+02±1069.3	4.24E+02±1017.1	9.51E+02±2182.9	3.62E+02±604.2	2.65E+01±46.8	3.75E+03±7983.5	N.D	8.70E+03±17528.1	4.10E+02±719.9	8.50E+00±20.3	2.25E+01±38.5	3.45E+01±41.7	3.78E+02±214.5	--

Table (3): The Average and ± standard deviation (STD) for some Dyes degrading bacteria for five textile factories and environmental samples

Parameters(CFU/mL)	Average ± STD												
	Factory 1		Factory 2		Factory 3		Factory 4		Factory 5		River Nile	Ismailia canal	El-Rahawy drain
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent			
Reactive blue	2.40E+03±3816.2	1.48E+03±1521.5	1.84E+03±1329.8	4.07E+03±2663.6	3.47E+03±542.8	5.14E+03±2353.3	1.08E+03±1449.6	8.38E+02±1654.1	1.54E+03±2511.7	1.02E+02±152.9	1.70E+03±1092.2	1.28E+03±1298.7	3.10E+03±2076.7
Congo red	1.97E+03±1941.8	1.86E+03±1544.8	2.83E+03±776.3	3.90E+03±2621.5	2.65E+03±1533.8	5.05E+03±2825.4	7.08E+02±1078.5	3.27E+02±296.7	3.42E+02±716.5	5.24E+02±1213.2	9.38E+02±1188.2	1.51E+03±1176.9	2.66E+03±1793.1
Methyl red	1.78E+03±1376.1	1.35E+03±1393.4	2.67E+03±1688.5	2.20E+03±1075.2	2.77E+03±1721.8	4.35E+03±2256.3	8.57E+02±1262.7	2.45E+03±2648.8	4.22E+02±969.4	5.87E+02±977.8	8.87E+02±1283.2	1.29E+03±1240.5	3.55E+03±1396.4
Reactive red	2.04E+03±2724.3	9.33E+02±1202.8	2.59E+03±1495.2	2.53E+03±1805.	3.52E+03±746.8	4.28E+03±2339.6	9.73E+02±1394.6	5.66E+02±1084	5.83E+02±718.9	2.06E+02±438.9	2.09E+03±1541.8	2.13E+03±1174.2	1.03E+04±18522.2
Direct red	2.23E+03±2173.2	1.40E+03±1361.1	1.90E+03±1391.5	2.02E+03±767.9	3.83E+03±950.1	4.73E+03±1618.2	6.49E+02±1316.7	6.10E+02±1280.7	4.10E+02±719.9	2.82E+01±32.9	9.53E+02±1243.8	1.46E+03±1356.2	3.18E+03±1787.7
Direct blue	3.13E+03±2758.9	8.33E+01±144.3	2.37E+03±1255.9	2.12E+03±1075.9	2.96E+03±1817.4	3.30E+03±2181.7	1.04E+02±107.1	2.29E+02±259.8	3.68E+02±616.8	3.17E+02±661.9	7.48E+02±758.4	7.58E+02±714.5	3.90E+03±1358.9
Crystal violet	7.25E+03±7424.5	8.36E+02±1041.56	7.31E+02±1748.7	1.14E+03±1993.2	3.42E+02±608.4	1.97E+03±3802.5	4.90E+02±1067.9	2.00E+01±21.2	2.00E+01±44.7	1.20E+00±2.7	1.93E+02±254.7	2.41E+02±390.2	6.90E+02±614.6
Fast green	1.48E+04±29463.5	1.00E+02±164.6	1.52E+03±1211.9	2.63E+03±1920.1	3.55E+03±1444.6	3.20E+03±1432.5	5.92E+02±1290.5	2.45E+03±3489.1	7.50E-01±1.5	1.84E+01±40	2.50E+02±273	5.74E+02±945.8	3.25E+03±506.6
Malachite green	3.07E+03±2733.8	9.00E+02±1389.2	2.87E+03±2735.8	2.60E+03±1885.7	3.60E+03±2241.4	3.50E+03±1682.9	6.06E+02±1230.5	5.91E+02±1290.6	5.00E-01±0.58	7.25E+00±8.8	6.03E+02±1001.8	1.10E+03±1685.8	1.55E+03±1628.3
Methylene blue	2.75E+03±2678.2	4.07E+02±352.8	1.04E+03±637.8	2.33E+03±1276.9	3.57E+03±1433.4	3.77E+03±1197.8	6.70E+02±1470.4	8.08E+02±1471	2.72E+01±52.3	4.21E+02±1018.4	6.40E+02±994.2	4.09E+02±836.6	2.92E+03±1499
Mix	2.04E+04±26537.2	7.50E+02±401.1	7.16E+02±1049.7	1.89E+03±1243.9	2.67E+03±838.3	2.30E+03±1200	4.50E+02±772.9	1.78E+03±2269.8	1.35E+02±288.8	8.00E+00±17.9	1.56E+02±296.5	2.02E+02±301.1	2.20E+03±1271.4

Table S1. Standard EC50 percent and toxicity level (Source: Modern Water Incorporation (2016)

EC50% Degree	Toxicity Level
0–19	Extremely toxic
20–39	Very toxic
40–59	Toxic
60–79	Moderately toxic
≥100	Nontoxic and safe

Table (4): some heavy metals from textile wastewater collected from five textile factories and environmental samples

Heavy metals (mg/l)	Factory 1		Factory 2		Factory 3		Factory 4		Factory 5		River Nile	Ismailia Canal	El-Rahawy
	before treatment	after treatment	before treatment	after treatment	before mixing	after mixing	before mixing	after mixing	before mixing	after mixing			
Iron (Fe)	1.5	1	0.6	0.4	0.4	51.5	0.5	0.76	0.7	51.5	0.19	0.09	2.85
Manganese (Mn)	0.03	0.03	3.4	0.4	0.45	0.2	0.17	0.24	0.02	0.2	0.04	0.03	0.17
Copper (Cu)	0.29	0.15	< 0. 01	0.03	< 0. 01	0.07	<0.01	< 0. 01	0.02	0.07	< 0. 01	< 0. 01	< 0. 01
Zinc (Zn)	0.34	0.17	0.08	0.03	0.12	0.06	0.06	0.5	0.8	0.06	<0.01	<0.01	3.33
Arsenic (As)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.02	<0.001	< 0.001	0.02	0.02	< 0.001	< 0.001	0.03
Lead (Pb)	<0.001	< 0.0 01	<0.001	< 0.001	< 0.001	<0.001	<0.001	< 0.001	<0.001	<0.001	<0.001	< 0.001	0.35
Chromium (Cr)	< 0.001	0.03	< 0.001	0.03	< 0.001	0.03	<0.001	< 0.001	<0.001	0.03	<0.001	< 0.001	< 0.001
Cadmium	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	<0.001	<0.001	< 0.001	<0.001	<0.001	<0.001	< 0.001	< 0.001
Nickel (Ni)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.2	<0.001	< 0.001	0.06	0.2	<0.001	< 0.001	0.02
Molybdenum (Mo)	< 0.01	< 0.001	< 0.01	< 0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Mercury (Hg)	<0.001	< 0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001
Cobalt (Co)	< 0.01	< 0.01	< 0.01	< 0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

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