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Chemical and Microbiological Analyses of Certain Water Sources and Industrial Wastewater Samples in Egypt

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Abstract: The chemical analyses included quantitative measurement of electrical conductivity, alkalinity, hardness sulphate, pH, total dissolved solids, chloride, as well as dissolved oxygen and BOD were carried out. The microbiological examination for different water sources and industrial wastewater samples was also conducted. Some of heavy metals, Co^{2+}, Cu^{2+}, Fe^{3+} and Mn^{2+} were determined in fresh water, while other metals, such as Cr^{6+}, Co^{2+}, Zn^{2+} and Ni^{2+} were measured in industrial wastewater. Results of the chemical analysis showed that all measured parameters were found within the limitation either national or international law, except some samples which showed higher values than the permissible limits for some measured parameters. The microbiological analysis exhibited presence of yeasts, fungi and bacteria. Most bacterial isolates were short rod, spore formers as well as coccoid shaped bacteria. The efficiency of water treatment process on the reduction of microbial load was also calculated. Regarding the pathogenic bacteria, data showed that neither water samples nor industrial wastewater contain pathogens when using specific cultivation media for the samples examination. Furthermore, data proved the possibility of recycling of the tested industrial wastewater on which some microorganisms can grow. Data showed also that the percent of heavy metals removal can reach to more than 70% in some cases as a result to bacterial treatment of industrial wastewater.

Key words: Industrial wastes, chemical pollutants, proteolytic bacteria, lipolytic activity, heavy metal removal by microorganisms.

Introduction

Biotechnology for hazardous waste management can be defined as the development of systems that use biological catalysts to degrade or detoxify environmental pollution. The water that the employees drink must meet public health standards from both chemical and microbiological point of view. This can achieve when the water source tested by recommended methods described in Standard Methods for the Examination of Water and Wastewater (APHA, 1992). The bacteriological standards for drinking water that coliform bacteria must not be present at levels indicating contamination of the water by sewage. Total plate counts of the water sometimes are made to indicate when trouble may be incipient so that such trouble can be forestalled. Unfortunately, in Egypt, the river Nile receives heavy load of wastes and effluents particularly from developing industries and agriculture as well as domestic discharges. In Dakahlia Governorate, industrial wastewater from Oil and Soap Company (Sandoup), Fertilizer Factory (Talkha) and Fodder Factory (Sherbin) discharge canals directly attached to the river Nile. Therefore, they are considered as serious pollution sources for drinking water. Agricultural, industrial and domestic wastes are also the major sources of surface water pollution with heavy metals (Polprasert, 1982). The use of microorganisms to remove or to reduce of heavy metals from aqueous solution, wastewater as well as industrial effluents has been examined by many investigators (Yazgan and Ozcengiz, 1994). The purpose of this investigation is to examine some sources of either drinking water or industrial waste water in Dakahlia Governorate (Nile Delta, Egypt) from both chemical and microbiological view point.

Materials and Methods

Water samples: Water samples were collected from four different water supplies in Dakahlia Governorate. The first is the water treatment plants (WTP) of Sherbin and Bossat Kareem El-Dein. The second is the compact units of two different locations namely Gamasa (unit 2) and Fasco (unit 4).

Wastewater samples: Wastewater samples were collected from Fodder Factory of Sherbin (FFS), Fertilizer Factory of Talkha (FFT), Oil and Soap Company of Sandoup (OSC), sludge from water treatment plant of Sherbin (WTP), the outlet of electrical power plant (EPP) and El-Nasr Company for Particle Board and Resin (PBR). All samples were collected in 5 littre polyethylene bottles for the chemical analysis and one liter sterile glass bottles for the microbiological examination. The procedure was carried out according to Standard Methods for the Examination of Water and Wastewater (APHA, 1992).

Chemical analysis: The pH values were measured by HANNA instrument, HI931401 Microprocessor pH meter. Both conductivity and TDS were measured using OAKLON, TDS/conductivity meter. Chloride, sulfate, alkalinity, hardness, dissolved oxygen and BOD were determined as described in the Standard Methods for the Examination of Water and Wastewater (APHA, 1992). The concentration of metals in all tested samples was determined by using 2380-Perkin-Elmer flame atomic absorption spectrophotometer following the recommended standard operating procedure. BDH standard solution used and internal standards were applied to check for interference problem. The metals concentration was determined in triplicate and the mean value was recorded. All chemicals used were of high grade. This measurement was also done according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1992).

Microbiological procedures

Cultivation media used: The following general and specific cultivation media were used for different purposes according to the Bacteriological Analytical Manual for Foods (FDA, 1976). Tryptone glucose yeast agar and nutrient agar (TGY & NA), were used for isolation and total counting of the microbial load of the tested samples. Milk agar (MA), was used for isolation and counting of proteolytic bacteria. Fuchsin lactose agar (Endo C agar) was used to detect the bacterial members of the

Enterobacteriaceae. Mannitol, sodium chloride phenol red agar (MSC), was specifically used for detection of *Staphylococcus* sp. Sabouraud glucose agar medium (SGA) was selectively used for *Enterococcus*. Bactoagar F-medium (BFA) is recommended for the detection of *Pseudomonas spp.* Nutrient agar containing olive oil was used for isolation and counting the lipolytic bacteria. The composition of these culture media was as described in Oxoid (1982).

Isolation and purification of microorganisms from tested samples: One ml sample of examined industrial wastewater dispersed in appropriate volume (9 ml) of distilled water and different serial dilutions were made with vigorous shaking. The original sample was used as it is in case of drinking water. One-ml sample was then taken and plated on different cultivation media and appropriate periods of incubation at 37°C according to the purpose required as mentioned above. From the preceding step, well-separated colonies were selected and transferred to the slope agar. In order to make sure the purity of these isolates, each of them was suspended in sterile water and recultivated again for two cycles followed by single colony isolation after streaking onto plates. Slope cultures obtained were considered pure when they showed uniform morphological feature by microscopic examination. Preliminary identification of the three isolates was achieved by Gram reaction, spore formation, motility, capsule formation, presence of water-soluble pigments followed by biochemical tests recommended by Sneath *et al*. (1986). Five bacterial isolates namely; *Bacillus sp.* FFT50, *Pseudomonas sp.* OSC15, *Bacillus sp.* FFS6, *Bacillus sp.* WTP30, *Pseudomonas sp.* EPP22 and *Pseudomonas sp.* PBR4 were selected and maintained on NA slant at 5°C till use.

Bacteriological treatment of wastewater: The tested wastewater was diluted to 50% by distilled water on which the selected bacterial strains allow to grow. Each strain was grown on a correspondence waste for 36 hr incubation period at 37°C. The bacterial growth was then enzymatically detected. The casein digestion method was followed for quantitative measurement of proteinase activity together with the cells denisities (OD, 600 nm). A unit of proteinase as tyrosine unit (TU/ml cultural filtrate) was defined as that quantity of enzyme, which produce TCA-soluble fragments, giving blue colour equivalent to 1 µg tyrosine under the assay conditions (Lupin *et al*., 1982). Lipase activity was titrimetrically determined. The reaction mixture contained 5.0 ml of 5% olive oil emulsion in 7% gum acacia, 5.0 ml of 0.2 M tris-HCl buffer (7.5), 0.2 M $CaCl_2$, 1.0 ml of enzyme solution and 2.0 ml of distilled water. After one-hour incubation, the total amount of liberated fatty acids was titrated against N/100 NaOH. The same procedure was followed with boiled enzyme solution as a control (Chopra and Chander, 1983).

Results and Discussion

Chemical properties

Fresh water: As shown in Table 1, it is clear that the pH values of all raw and treated water samples have a slight basic effect. These results suggest that pH values of raw water were slightly affected by the treatment adopted. All these values were found within the national and international limits, since the limitation of pH values of drinking water is 6.0-8.5 (WHO, 1993). According to WHO recommendation, the total dissolved solids (TDS) in drinking water must not increase over 500 mg/L. The values of TDS obtained of tested water either raw or treated samples are within this figure reveal the suitability of the TDS concentration in this type of water. Alkalinity of water is the sum of all the titratable bases. It is a measure of an aggregate property of water and it is significant in many uses and treatments of natural waters and wastewaters. Waters with the concentration of sulphate ions more than 250 mg/L may be destructive to concrete structures owing to the formation of gypsum. The presence of chlorides and sulphates in water in appreciable concentrations is indicative of an elevated solute residue which makes the water poorly suitable for feeding of stem boiler plants or as process water in the manufacture of certain polymers, synthetic rubber, some grades of paper, etc. The water hardness property is due mainly to the presence of carbonates, bicarbonates, sulphates, or other compounds of calcium and magnesium. Magnesium and calcium sulphates and chlorides are responsible for non carbonate hardness of water. Calcium compounds are the most common source of hardness in water supplies as they readily dissolved from limestone deposits. Results in Table 1 showed that Ca^{++} is higher than Mg^{2+} by more than two times. Hardness in water supply is not dangerous to health, but is that hardness diminishes very considerably the capacity of water to form lather with soap. All examined samples are within the permissible limits of WHO and Egyptian Standards as well (500 mg/L).

Some of soluble heavy metals were also measured (µg/L) and their values recorded in Table 1. The concentration of copper in water is usually attributed to the corrosive action of copper salts as a result of the activity of the aquatic organisms. Manganese is also considered to be one of the essential elements in organic matter, which greatly influence on its occurrence in natural water (El-Sharouny, 1989).

Wastewater: The physico-chemical analysis of tested wastewater are illustrated in Table 2. Data show that all the tested samples have the same basic effect of pH, but it is also within the national and international limits of pH values, except the sample of fertilizer factory of Talkha, which have high pH value being 11.2 which exceeds the permissible limit. In case of TDS and conductivity, the sample of OSC is the highest sample being 2300 mg/L and 4600 µs/cm, respectively. Again the OSC sample gave the highest value in case of sulphate and chloride being 322.5 mg/L and 1349.7 mg/L, respectively.

Microbiological values

Fresh water: The microbiological data express in colony forming unit per ml (cfu/ml) of examined fresh water samples are listed in Table 3. From tabulated data, it could be noticed that the efficiency of microbial count reduction in Sherbin WTP is much higher than that of Bossatt. For example, the total bacterial count using TGY medium (70% spore formers) decreased by 48.1 and 22.7% in case of Sherbin WTP and Bossatt WTP, respectively. In case of lipolytic bacteria (79% short rods), the reduction percentages were 39.2 and 25.1% for the two mentioned samples, respectively.

About 85% of the total proteolytic bacteria (MA medium) was found to be spore-forming bacteria. The reduction of this count was reached to 30.4 and 32.8% for Sherbin and Bossat samples, respectively. When using SGA medium, the observed fungi was about 65%, while yeast number represented about 30%. By the microscopic examination, most present fungi were *Aspergillus spp., Penicillium spp.* and *Alternaria spp.*, while, *Sacchoromyces spp.* and *Candida spp*. were the yeast present. The biotreatment process leads to decrease this count to 68.2% in case of Sherbin, while 37.6% was detected with Bossat sample. Concerning the compact unit (CU), the unit of Fasco showed to be more efficient than that one of Gamasa in all microbial group, except the proteolytic bacteria (MA) and fungi (SGA) as shown in the same Table. The viable count of *Pseudomonas spp.* (BAF) came in second order in case of Sherbin sample (WTP), Gamasa (CU) and Fasco (CU), but it was in the first with the sample of Bossat

Table 1: Physico-chemical properties and heavy metal analysis of fresh water samples.

Measured parameters	Water sources (WS)							
	Water treatment plant (WTP)				Compact unit (CU)			
	Sherbin		Bossatt		Gamasa		Fasco	
	R	T	R	T	R	T	R	T
pH	7.9	7.6	8.0	7.8	7.9	7.3	8.0	7.3
TDS, mg/L	222.0	224.0	217.0	220.0	169.0	181.0	176.0	180.0
EC, µs/cm	444.0	448.0	434.0	440.0	338.0	362.0	352.0	360.0
Alkalinity, mg/L:								
HCO^-_3	192.3	184.6	200.0	180.0	173.1	150.0	177.0	153.5
Total	192.3	184.6	200.0	180.0	173.1	150.0	177.0	153.5
Hardness, mg/L:								
Ca^{2+}	46.7	46.7	42.0	42.0	37.4	37.4	43.9	43.9
Mg^{2+}	18.9	18.9	17.0	17.0	14.9	14.9	13.2	13.2
Total	194.2	194.2	174.8	174.8	155.3	155.3	190.3	190.3
SO_4^-, mg/L	35.0	59.5	40.1	53.7	32.3	59.4	32.7	56.4
Cl^-, mg/L	52.0	60.0	50.0	54.0	40.0	46.0	40.0	44.0
Heavy metals, µg/L:								
Co^{2+}	16.0	35.0	16.0	29.0	13.0	10.0	13.0	13.0
Cu^{2+}	7.2	10.8	7.1	6.1	5.9	6.5	5.1	5.8
Fe^{3+}, Fe^{2+}	89.0	76.0	77.0	67.0	80.0	78.0	72.0	74.0
Mn^{2+}	99.0	53.0	99.4	94.6	60.0	82.0	73.0	70.0

R: Raw T: Treated.

Table 2: Physico-chemical properties of industrial wastewater samples.

Chemical analysis	Fertilizer factory FFT	Oil & Soap Company OSC	Fodder Factory FFS	Sludge of WTP WTP	Electrical Power Plant EPP	El-Nasr Company PBR
pH	11.2	7.53	7.8	8.26	7.85	10.83
TDS, mg / L	283.0	2300.00	11.4	191.00	186.00	859.00
Conductivity, µs/cm	566.0	4600.00	22.7	382.00	372.00	1718.00
Alkalinity, mg/L						
OH	48.0	0.00	0.0	0.00	0.0	0.00
ph ph	184.0	0.00	0.0	0.00	0.0	0.00
HCO^-_3	--	--	--	--	--	460.00
CO^{2-}_3	272.0	148.00	72.0	188.00	150.0	260.00
Total	320.0	148.00	72.0	188.00	150.0	720.00
Hardness, mg/L						
Ca^{2+}	66.2	35.80	0.0	37.80	31.49	8.02
Mg^{2+}	0.0	4.00	4.7	16.10	14.48	12.16
Total	165.1	399.90	19.4	155.30	138.10	70.00
Sulphate, mg/L	47.6	322.50	0.0	39.50	24.80	43.00
Chloride, mg/L	75.99	1349.70	14.0	39.99	31.24	179.95
Dissolved oxygen, mg/L	8.0	0.00	7.3	8.70	8.00	9.04
BOD	2.51	21.98	ND	9.69	6.39	40.48

ND: Not determined.

Table 3: Different microbial groups in examined treated fresh water.

Cultivation media used*	Colony forming unit											
	Water treatment plant (WTP)						Compact unit (CU)					
	Sherbin			Bossat			Gamasa			Fasco		
	R	T	E%	R	T	E%	R	T	E%	R	T	E%
TGY	145.0	75.3	48.1	110.4	85.3	22.7	210.0	165.0	21.4	285.2	104.3	63.4
OA	25.5	15.7	39.2	40.6	30.4	25.1	65.3	40.2	38.4	88.6	39.2	55.8
MA	45.6	18.4	59.7	55.5	35.2	36.6	73.8	33.4	54.7	95.9	77.3	19.4
BAF	130.4	90.8	30.4	156.6	105.3	32.8	110.3	85.7	22.3	125.5	70.4	43.9
SGA	95.4	30.3	68.2	105.7	65.7	37.6	75.1	40.3	46.3	100.2	66.4	33.7

*See materials and methods R : Raw water T: Treated water E: Efficiency %

Table 4: Microbiological examination of some industrial wastewater samples obtained from different industries at Dakahlia governorate.

Source of wastewater	Examined microbial groups (cfu/ml x 10^3)							
	Total count		Special groups				Fungi & yeast	
	NA	TGY	OA	MA	SA	BFA	SGA	
Fertilizer Factory (Talkha)	90.4	165.3	50.9	85.9	34.6	120.4	60.5	45.8
Oil & Soap Company (Sandoup)	180.6	210.5	140.3	60.4	22.8	180.8	75.8	15.9
Fodder Factory (Sherbin)	215.8	260.2	85.9	160.3	30.4	210.7	32.9	24.6
Sludge of WTP (Sherbin)	205.2	245.6	105.2	180.2	15.3	260.3	80.6	90.4
Electrical Power Plant	95.9	115.7	40.6	40.7	18.9	95.1	20.3	15.7
El-Nasr Co. for Particle Board and Resin	170.9	198.5	77.8	13.9	7.5	45.3	3.6	5.3

Table 5: Some bacterial enzymes obtained in some industrial wastewater used as cultivation media.

Wastewater source	Bacterial strain used	Proteinase activity TU/ml/min.			Lipase activity 0.01 N NaOH/ml/10 min.		
		After incubation period (hr) of					
		12	24	36	12	24	36
Fertilizer Factory (Talkha)	*Bacillus sp.*, FFT 50	22.0	36.4	65.3	32.8	38.6	49.2
Oil & Soap Co. (Sandoup)	*Pseudomonas sp.*, OSC15	28.4	39.3	58.3	40.0	55.6	72.4
	Bacillus sp., FFS6						
Fodder Factory (Sherbin)	*Bacillus sp.*, WTP30	82.6	112.4	125.6	38.2	51.8	64.9
Sludge of WTP (Sherbin)	*Pseudomonas sp.*, EPP22	45.0	60.5	99.0	24.8	32.6	45.4
Electrical Power Plant	*Pseudomonas sp.*, PBR4	13.2	18.3	36.5	2.8	8.6	15.2
El-Nasr Co. for Particle Board and Resin		8.3	14.8	28.7	5.3	12.4	16.8

Table 6: Effect of bacterial treatment on metal ions concentration in industrial wastewater.

Wastewater source		Heavy metal conc., mg/L			
		Cr^{6+}	Co^{2+}	Zn^{2+}	Ni^{2+}
Fertilizer Factory (Talkha):			*Bacillus sp.* FFT50		
	Before	0.27	0.220	1.074	0.12
	After	0.24	0.196	0.232	0.09
	Fold	1.13	1.122	4.63	1.33
	Percent	11.11	10.91	78.40	25.0
Oil & Soap Company (Sandoup):			*Pseudomonas sp.* OSC15		
	Before	0.27	0.215	0.940	0.14
	After	0.20	0.186	0.564	0.11
	Fold	1.35	1.39	1.67	1.27
	Percent	25.93	13.49	40.0	21.43
Fodder Factory (Sherbin)			*Bacillus sp.* FFS6		
	Before	0.27	0.202	1.17	0.13
	After	0.17	0.167	0.020	0.09
	Fold	1.59	1.21	58.45	1.44
	Percent	37.04	17.73	98.29	30.77
Sludge of WTP (Sherbin)			*Bacillus sp.* WTP30		
	Before	0.027	0.023	0.531	0.012
	After	0.023	0.018	0.259	0.010
	Fold	1.17	1.28	2.05	1.20
	Percent	14.82	22.12	51.22	16.67
Electrical Power Plant:			*Pseudomonas sp.* EPP22		
	Before	0.27	0.192	0.96	0.112
	After	0.18	0.189	0.77	0.112
	Fold	1.50	1.02	1.24	1.000
	Percent	33.33	1.56	19.25	0.00
El-Nasr PBR:			*Pseudomonas sp.* PBR4		
	Before	0.156	ND	1.225	ND
	After	0.095	ND	0.655	ND
	Fold	1.23	ND	1.87	ND
	Percent	39.10	ND	46.53	ND

ND = Not determined.

Kareem El-Dein (WTP). The efficiency of the number seems to be closed in case of WTP, but highly varied with CU as shown in the same Table 4. Microbiological examination revealed that no pathogenic bacteria were detected in all tested samples. This may be ascribed to non recent sewage effluents entry or present at the time of sampling either in raw or treated water samples. These results are not in agreement with those previously obtained by Hosny *et al*. (1990), who found faecal coliform and *Streptococcus faecolis* in Nile water.

Industrial wastewater: Data in Table 4 showed that the sample of Fodder factory of Sherbin was heavily polluted by the total microbial count when using TGY medium followed by sludge of water treatment plant (WTP) of Sherbin, Oil and Soap Company of Sandoup and Al-Nasr Company for Particle Board and Resin. The Fertilizer factory of Talkha and the outlet waste of the electrical power plant came last being 165.3 and 115.7 cfu x 10^3/ml, respectively. Regarding *Pseudomonas spp.* (BAF medium), the highest count was recorded with sludge of WTP of Sherbin, then sample of Oil and Soap Company (Sandoup) and Fodder Factory (Sherbin). For the proteolytic bacteria (MA medium), sludge sample of WTP (Sherbin) was more polluted than the other tested samples followed by the Fodder factory of Sherbin. On the other hand, the lipolytic bacteria and fungal count present in examined samples showed to be less as shown in the Table 5.

Recycling of wastewater

Monitoring of bacterial growth: The isolated bacteria were again allowed to grow on the 50% diluted industrial wastewater obtaining different extracellular enzymes according to the chemical composition of these wastes. Table 6 illustrate the values of both proteolytic and lipolytic enzymes obtained during 36 hr incubation period at 37°C. The bacterium *Bacillus sp.* FFS6 showed to be in the first order followed by *Bacillus sp*. WTP30 regarding the proteinase production giving 125.6 and 99.6 TU/min/ml. cultural filtrate after 36 hr. The lowest value of this enzyme was detected by *Pseudomonas sp*. EPP 22, which was equal to 36.5 TU/min/ml cultural filtrate. For the production of lipolytic activity, *Pseudomonas sp.* OSC15 was better, since it gave 72.4 LU/10 min/ml., while 64.9 lipase unit was obtained by *Bacillus sp.* FFS6 after 36 hr. incubation. Again the lowest lipase production was detected by the bacterium *Pseudomonas sp.* EPP22 after the same time of incubation period being 15.2 LU/10 min/ml. Obtained results proved that the survival of microorganisms in wastewater varied according to the chemical composition, pH, temperature and suspended organic matter. All these factors affecting the microbial enzyme production.

Reduction of heavy metals concentration: Results recorded in Table 6 demonstrated the effect of bacterial treatment on metal ions concentration. Tabulated data exhibited that *Pseudomonas sp.* PBR 4 removed 39.1% of Cr^{6+} found in PBR sample followed by *Bacillus sp.* FFS6 which removed about 37% of Cr value present in Fodder Factory of Sherbin followed by 33% removed by *Pseudomonas sp.* EPP22. The removal percent of the sample of Oil & Soap Company reached 25.9% by *Pseudomonas sp.* OSC15. For Co^{2+}, the highest removal percent are 22 and 17.7% achieved by *Bacillus sp.* WTP30 and *Bacillus sp.* FFS6, respectively. Interestingly, high percent of Zn^{2+} removal was achieved by all bacterial isolates used equal to 98.3% by *Bacillus sp.* FFS6, while 78.4% observed with *Bacillus sp.* FFT50. Thirty percent of Ni concentration was removed by *Bacillus sp.* FFS6, while 25% came next by *Bacillus sp.* FFT50.

Many strains of *Arthrobacter spp.* diffused in the soil and environment have been proved to be effective in heavy metal capture, such as Cd, Cr, pb, Cu and Zn (Grappeli *et al*., 1992). The removal of trace Cd (II) from aqueous solutions by compact pellets of *Rhizopus oryzae* was also established as reported by Huang and Chiu (1994). Common filamentous fungi in a biomass form to remove ions, such as Cd, Cu, pb and Ni from contaminated wastewater (Kapoor and Viraraghavan, 1995). Lead ions were also removed by *Pseudomonas sp.* in aqueous effluents as reported by Panchanadikar and Das (1994).

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