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Isolation of Heavy Metal Tolerant Fungi from Industrial Discharge

Fatma Migahed, Gehad Fawzy [*], Ahmed Abd Elrazak

[1] Botany Department, Faculty of Science, Mansoura University, Egypt

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Abstract: Industrial Discharge Effluent samples in addition to sediments near to discharge pipes were collected from four factories located at Mansoura city, Dakahlia governorate including ; Delta fertilizer factory (DFF), Talkha electric power plant (TEP), Marble and Granite factory (MGF) and Sandoup oil and soup factory (SOSF). Effluent samples were analyzed for physicochemical parameters, heavy metal concentration and mycological analysis. Among the heavy metals Chromium and Lead ions showed a level higher than WHO guideless. Fifty five and Fifty six resistant fungal isolates respectively were isolated from the collected sediments and discharges of the four factories through the four seasons. The Minimal Inhibitory Concentrations for the resistant isolates were determined and the most potential resistant fungal isolates were identified. The most resistant isolates were found to belongs to genera *Zygomycetes* , *Ascomycetes* and *Deutromycetes.*

Keywords: *Industrial Discharges, Heavy metal resistance, Soil and Water Fungi.*

INTRODUCTION

The River Nile is the crucial source of life in Egypt; as it is the main source of freshwater for domestic, irrigation, industry, a source of power from the hydroelectric generation facility at Aswan, and a mean of transportation for people and goods [1].

The River Nile arrives Egypt at Adindan village of Nubia (Start location of lake Nasser) and routes northward till Cairo for approximately 1188 Km. Starting at Cairo it moves northwestward for a distance of nearly 23 Km where Nile delta starts and the river rifts into the Damietta (eastern) branch (about 242 Km) and the Rosetta (western) branch (about 236 Km) [1]. By the commencement of fifties of the 20^{th} century, heavy industrialization was launched in Egypt along the river Nile, in Delta, Cairo and Alexandria. Chemicals, food, metal products and textiles industries were the most major activities in Egypt [2]. Certainly, the influence of the industrial pollution in Egypt affects all environmental aspects including air, water and land. Industrial pollutants contaminating the surface water caused deleterious effects on structure and function of resident biological communities.

The water quality measurement consists of several parameters that could be measured using proper methods. Some simple measurements such as temperature, pH, dissolved oxygen and conductivity. Wastewater quality could be defined by physical, chemical, and biological characteristics. Chemical parameters associated with the organic content of waste water include the biochemical oxygen demand (BOD) and chemical oxygen demand (COD). Inorganic chemical parameters include hardness, pH, chlorides, sulfates, sodium, potassium, heavy metals (lead, chromium, copper, cobalt, manganese, cadmium, iron and zinc), nitrogen (organic, ammonia, nitrite, and nitrate), and phosphorus [3].

Heavy metal pollution is one of the most important environmental problems today; heavy metals are defined as metals with a specific weight usually more than 5.0 g/cm^3, which is five times higher than water. The toxicity of heavy metals occurs even in low concentrations of about 1.0-10 ppm [4]. Heavy Metal Contamination is a general term given to describe a condition having abnormally high levels of toxic metals in the environment. Heavy metals are subtle, silent, stalking killers. It was realized that sometimes the natural cycles can pose a hazard to human health because the level of heavy-metals exceed the body's ability to cope with them. The situation becomes worst by the addition of heavy-metals to the environment as a result of both the rapidly expanding industrial and domestic activities. The metals are introduced into the environment during mining, refining of ores, combustion of fossil fuels, industrial processes and the disposal of industrial and domestic wastes [5]. Human activities also create situations in which the heavy-metals are incorporated into new compounds and may be spread worldwide [6].

The health hazards presented by heavy-metals depend on the level of exposure and the length of exposure. In general, exposures are divided into two classes: acute exposure and chronic exposure. Acute exposure refers to contact with a large amount of the heavy-metal in a short period of time. In some cases the health effects are immediately apparent; in others the effects are delayed. Chronic exposure refers to contact with low levels of heavy-metal over a long period of time [6].

Toxic effects of heavy metals on human safety are very well known: negative effects on metabolism, damages to correct functioning of the cells, with tumors [7], and mutations [8] developments [9]. There is overall potential to be toxic even at relatively minor levels of exposure. The toxicity of metals most commonly involves the brain and the kidney but other manifestations occur and some metals, such as arsenic are clearly capable of causing cancer. An individual with metals toxicity, even if high dose and acute, typically has very general symptoms, such as weakness or headache [10].

Thus, the monitoring on the level of trace metals in wastewater must be performed by obeying Environmental Quality (Sewage and Industrial Effluents) Regulations 1974 [11], in order to control the level of trace metals in wastewater before it is being discharged from factories to the environment and subsequently the environmental pollution that may affect human health could be minimized. Similar studies were carried out by other researchers by using different analytical methods, sample preparation methods and types of samples tested.

The most commonly used analytical methods in heavy metal determination are atomic absorption methods which are simple yet affordable methods, followed by the chromatographic methods. In the determination of heavy metals, pre-concentration and separation methods have been routinely used to cope with low metal levels. Different pre-concentration techniques for heavy metals such as cloud point extraction [12], solid phase extraction [13] and chelating agent preconcentration [14] were performed by researchers before the samples being analyzed by flame atomic absorption spectroscopy. Types of sample that are often tested by researchers for heavy metal determination are environmental samples [15], food samples [16, 17], biological samples [18, 19] as well as plastic materials [20, 21]. Most of the methods and procedures used by the researchers are able to give reliable results on determination of heavy metals, but they require either extensive and complicated procedures or the utilized methods are too time-consuming.

The introduction of heavy metal compounds into the environment generally induces morphological and physiological changes in the microbial communities [22], hence exerting a selective pressure on the microbiota [23]. Generally, the contaminated sites are the sources of metal resistant micro-organisms [24]. Fungi and yeast biomasses are known to tolerate heavy metals [25, 26]. They are a versatile group, as they could adapt and grow under various extreme conditions of pH, temperature and nutrient availability, as well as high metal concentrations [27].

El-Morsy [28] studied 32 fungal species isolated from polluted water in Egypt for their resistance to metals and found that *Cunninghamela echinulata* biomass could be employed as a biosorbent of metal ions in wastewater. Vadkertiova and Slavikova [22] have studied metal tolerance of yeasts isolated from polluted environments and found that there is an interspecific and intraspecific variation in the metal tolerance among tested strains. In the same way, Zafar *et al.* [29] reported promising biosorption for Cd and Cr by two filamentous fungi, *Aspergillus* sp. and *Rhizopus* sp., isolated from metal-contaminated agricultural soil. The present work reports the occurrence of metal-resistant fungi isolated from polluted industrial effluents and sediment samples.

MATERIAL AND METHODS

Sample collection: Water samples were collected from the industrial effluents discharge pipe of four different factories in Al Dakhliya governorate these are; Delta fertilizer factory (DFF), Talkha electric power plant (TEP), Marble and Granite factory (MGF) and Sandoup oil and soup factory (SOSF). In addition to water, the sediments near to the discharge pipes were collected and prepared as reported by Webster [30].

Physicochemical parameters of water: Ca^{++} , Mg^{++}, Na^{+}, ,SO_4, COD ,P , NO_3-N ,NO_2-N ,NH_4-N and CO_3^{--} were determined and also field measurements such as temperature , pH ,D.O, B.O.D and electrical conductivity were observed.

Heavy metals analysis: Heavy metals were determined using atomic absorption spectro-photometer type Buck scientific accusys 214/215 according to Wirsen [31].

Isolation of the heavy metals resistant fungi: To isolate the potential resistant fungi from the collected samples, one ml of each of the collected samples (water and suspension of sediment samples) from each season was inoculated to Potato Dextrose agar medium (PDA) which amended with 100 ppm of $Cr^{6+}$and 100 ppm of Pb^{2+} separately. Subsequently, the plates were incubated at 28°C for 7 days and the fungal colonies were collected, according to the morphological differences, sub-cultured, purified and were preserved on PDA slants for further work.

Determination of minimum inhibitory concentrations (MICs): The minimum inhibitory concentrations of the most frequent selected isolate to Cr^{6+}, Pb^{2+} were determined by the diffusion method. Metal ions were added separately to PDA medium at concentrations of 400 to 7000 ppm. The plates were inoculated with 8 mm agar plugs from young fungal colonies, pre-grown on PDA. Three replicates of each concentration and controls without metal were used. The inoculated plates were incubated at 25°C for at least 7 days. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of metal that inhibit visible growth of the isolate [32].

Identification of the most potential isolates: Potato Dextrose Agar (PDA) was used for culturing the proposed fungi; isolated fungi were identified based on the observation of cultural and morphological characteristics, color of colony and sporulation. Examination was carried out using needle-mount preparation whereby fragments of the sporing surface of the culture was taken. This was teased out in drop of alcohol on a cleaned glass slide using needle. The fragment was stained by adding a drop of lactophenol. A cover slip was applied carefully avoiding air bubbles and the preparation was examined under light microscope [33].

RESULTS AND DISCUSSION

Sample collection and water analysis: Water samples were collected from the industrial effluents discharge pipe of four different factories in Al Dakhliya governorate these are; Delta fertilizer factory (DFF), Talkha electric power plant (TEP), Marble and Granite factory (MGF) and Sandoup oil and soup factory (SOSF). In addition to water, the sediments near to the discharge pipes were collected and prepared as reported by Webster [30].

Heavy metal analysis in water: The water samples were analyzed for their total content of heavy metals, it is well known that a long-time exposure of water and sediment to heavy metals can produce considerable modification of their microbial populations, reducing their activity and their number [34]. Heavy metal (Pb^{+2} ,Cd^{+2} ,Ni^{+2} ,Fe^{+3} ,Cu^{+2} ,Cr^{+6} ,Zn^{+2} ,Co^{+2} and Mn^{+2}) concentrations were determined using Atomic Absorption Spectrometry (type Buck scientific accusys 214/215 according to Wirsen [31]. Heavy metals content of water samples is listed in Tables 1&2. The concentration of Cr^{+6}, Pb^{+2} in all water samples of the four factories was found to be above the permissible limits of 0.01 and 0.5ppm, respectively [35].

In autumn samples the concentration of Pb^{+2} ranged between 0.4224 ppm in TEP sample and 0.6652 ppm in MGF sample , for Cd^{+2} the concentration was non-detectable in MGF sample and 0.0289 ppm in TEP, for Ni^{+2} the concentration was non-detectable in TEP sample and 3.4631 ppm in SOSF sample.

For Fe^{+3} the concentration was ranges between 0.2014 ppm in SOSF sample and 0.3824 ppm in TEP sample, for Cu^{+2} concentration of it varied between 0.0059 ppm in MGF and 0.1525 in DFF ,for Cr^{+6} the concentration of it varied between 0.2131 ppm in DFF sample and 0.6960 ppm in SOSF, for Zn^{+2} the concentration of it ranged between 0.204 ppm in MGF sample and 0.306 ppm in SOSF sample , for Co^{+2} the concentration of it varied between 0.173 ppm in TEP sample and 0.914 ppm in DFF sample and finally the concentration of Mn^{+2} varied between 0.038 ppm in MGF sample and 0.216 ppm in SOSF sample as shown in (**Table 1**).

From (**Table 2**) the concentration of Cr^{+6}, Pb^{+2} in all water samples of the four factories were found to be above the permissible limits of 0.01 and 0.5ppm, respectively (35). So Cr^{+6}, Pb^{+2} were the two metals of our concern in this study and the concentration of them were detected in the other seasons also.

Table 1: Heavy metal analysis (ppm)for four investigated factories (Delta fertilizer factory (DFF), Talkha electric power plant (TEP), Marble and Granite factory (MGF) and Sandoup oil and soup factory) in autumn sample(screening of the most common heavy metals Pb ,Cd ,Ni,Fe,Cu,Cr,Zn,Co and Mn)

Heavy metals / Sites	Pb^{+2} ppm	Cd^{+2} Ppm	Ni^{+2} ppm	Fe^{+3} Ppm	Cu^{+2} ppm	Cr^{+6} ppm	Zn^{+2} ppm	Co^{+2} ppm	Mn^{+2} ppm
DFF	0.4528	0.0173	0.7611	0.3678	0.1525	0.2131	0.223	0.914	0.07
TEP	0.4224	0.0289	N.D	0.3824	0.0300	0.3879	0.225	0.173	0.06
MGF	0.6652	N.D	3.3050	0.2250	0.0059	0.5769	0.204	0.41	0.038
SOSF	0.5390	0.0248	3.4631	0.2014	0.0836	0.6960	0.306	0.90	0.216

ND: non detected

Table 2: Seasonal variation in Chromium and Lead (ppm) concentration of effluent samples for the four investigated factories (Delta fertilizer factory (DFF), Talkha electric power plant (TEP), Marble and Granite factory (MGF) and Sandoup oil and soup factory

Metal ions	Autumn				Winter				Spring				Summer			
	SOSF	DFF	MGF	TEP	SOSF	DFF	MGF	TEP	SOSF	DFF	MGF	TEP	SOSF	DFF	MGF	TEP
Chromium	0.69	0.21	0.57	0.38	0.042	ND	0.037	0.053	ND	0.068	0.016	ND	0.137	0.189	ND	0.701
Lead	0.53	0.45	0.66	0.42	0.158	0.4738	ND	0.34	ND	ND	ND	ND	ND	ND	ND	1.553

ND: non detected

In winter sample , Pb^{+2} concentration recorded higher value more than the permitted limit in DFF, SOSF and TEP samples, 0.4738 ppm ,0.158 ppm and 0.34 ppm respectively and in MGF sample was non detected while Cr^{+6} was not exceed permitted value .

In spring sample , Pb^{+2} was non detected in all four samples and Cr^{+6} was non detected in SOSF and TEP samples and Cr^{+6} was not exceed permitted value in MGF and DFF samples .

In summer sample, Cr^{+6} was recorded higher value more than the permitted limit in DFF, SOSF and TEP samples were 0.189 ppm ,0.137 ppm and 0.701 pmm respectively and was absent in MGF samples while Pb^{+2} was more than the permitted limit in TEP sample which recorded high concentration 1.553 ppm and non detected at the others as showing in (**Table 2**).

Physicochemical Parameter: Each water body has an individual pattern of physical and chemical characteristics which are determined largely by the climatic, geomorphologic, and geochemical conditions prevailing in the drainage basin and the underlying aquifer.

In the present study, the mean water temperature, pH, EC, DO and BOD of the selected sites (**Table 3**) were detected as there are important parameters that affect microbial populations.

Table 3: Seasonal variation in the field measurements of the effluent samples of the four investigated factories(Delta fertilizer factory (DFF), Talkha electric power plant (TEP), Marble and Granite factory (MGF) and Sandoup oil and soup factory) in temperature , EC ,pH , DO ,and BOD.

Items	Autumn				Winter				Spring				Summer			
	SOSF	MGF	TEP	DFF	SOSF	MGF	TEP	DFF	SOSF	MGF	TEP	DFF	SOSF	MGF	TEP	DFF
Temperature (°C)	30	30	50	40	25	19	40	38	25	22	35	31	28	26	40	35
EC (μs)	4200	843	771	1207	528	1510	950	1560	1730	1500	652	1210	4740	1420	388	1800
pH	7.4	7.93	8.09	7.72	7.89	7.68	7.8	7.8	7.214	6.583	6.654	10.405	7.25	8.22	8.3	9.5
DO (mg/l)	0.074	0.105	0.234	0.026	0.1105	0.134	0.143	0.139	0.019	0.113	0.208	0.1170	0.0065	0.151	0.243	0.0780
BOD (mg/l)	0.074	0.026	0.228	0.019	0.0976	0.1	0.198	0.1	0.19	0	0.273	0.098	0.0065	0.06	0.258	0.026

In the present study, the mean water temperature varied between 19°C in winter sample in MGF and 50°C in autumn sample in TEP (**Table 3**). The variation in the temperature of water samples was highly significant ($p < 0.001$) among different seasons and sites separately and season and site together (**Table 5**).

The values of pH of the selected sites (**Table 3**) were generally in the neutral and alkaline side; being ranged between 6.583 in MGF in spring sample and 10.4in DFF in spring sample also. However, pH values of water samples have no significant (**Table 5**). According to Samaan (1974) [36] and Kwaitkowski and Roff (1976) [37], the changes in pH are mainly due to photosynthesis activities of phytoplankton and aquatic plants, and respiration of animals and plants as well as variations in temperature.

The conductivity of most fresh water ecosystems ranges from 10 to 1,000 μs/cm but may exceed 1,000 μs/cm, especially in polluted waters. Conductivity measurements are useful in rivers for the management of temporal variations in total dissolved solids and major ions in this study electrical conductivity EC ranging from 388 μs in summer sample in TEP to 4200 μs in autumn sample in SOSF as showed in (**Table 3**), EC value have no significant (**Table 5**).

In this study, both seasonal variations of the DO in all sites were highly significant ($p < 0.01$). While DO in season have no significant and DO in site and season together show significant ($p < 0.05$) (**Table 5**). This is attributed to the activities of air movement which allow more transfer of oxygen across the air–water interface and also due to turbulence in the flowing water [38]. Relatively lower temperature in winter may increase the ability of water to hold dissolved oxygen [39] as showed in (**Table 3**) the DO ranged from 0.0065 mg /l in summer sample in SOSF to 0.243 mg /l in TEP in summer sample also.

In this study, the BOD values (**Table 5)** of the selected sites varied significantly ($p < 0.01$) among the investigated sites. Conversely, the seasonal variation was not significant while site and season together is significant ($p < 0.01$), BOD value varied between 0 mg/l in MGF in spring to 0.273 mg/l in TEP in spring sample also as showed in (**Table 3**).

Also chemical parameters (Ca^{++},Mg^{++}, Na^{+}, K^{+}, HCO^{3-},Cl^{-}, SO_4, CO^{3-}, COD ,DRP ,NO_3,NO_2 and NH_4) were analyzed for the effluent samples of the four investigated factories (Delta fertilizer factory (DFF), Talkha electric power plant (TEP), Marble and Granite factory (MGF) and Sandoup oil and soup factory) ,(**Table 4**) showed seasonal variation in the tested chemical parameters.

Extractable cations (Na^{+} and K^{+})In autumn sample Na+ ranged between 26.91 Meq / L in SOSF to 4.85 Meq / L in TEP while K+ ranged between 1.82 Meq / L in SOSF to 0.034 Meq / L in TEP ,in winter sample Na+ ranged between 10.18 Meq / L in DFF to 3.18 Meq / L in SOSF and K^{+} ranged between 0.51 Meq / L in MGF to 0.22 Meq / L in SOSF, in spring sample Na^{+} ranged between 11.47 Meq / L in SOSF to 4.10 Meq / L in TEP and K+ ranged between 0.72 Meq / L in sosf and 0.28 Meq / L in TEP and finally in summer sample Na+ ranged between 30.29 Meq / L in SOSF to 2.25 Meq / L in TEP and DFF and K+ ranged between 1.95 Meq / L in SOSF and 0.18 Meq / L in TEP and DFF (Table 4) with no significant as showing in (Table 5).

.Sulfate SO_4 in autumn sample ranged from 9.95 Meq / L in SOSF to 1.64 Meq / L in MGF, SO_4 in winter sample ranged from 3.74 Meq / L in DFF to 1.12 Meq / L in SOSF, in spring sample SO_4 ranged between 3.83 Meq / L in SOSF to 1.21 Meq / L in TEP, and finally in summer sample SO_4 ranged from 10.12 Meq / L in SOSF to 0.99 Meq / L in TEP and DFF (Table 4) with no significant as showing in (**Table 5**).

Chemical oxygen demand COD ,in autumn sample ranged between 9.75 ppm in SOSF and 6.14 ppm in TEP, in winter sample COD ranged between 29.6 ppm in DFF to 16.8 ppm in SOSF, in spring sample COD ranged between 33.8 ppm in SOSF and 18.9 ppm in TEP, finally in summer sample COD ranged between 59.8 ppm in SOSF to 17.9 ppm in TEP (**table 4**) COD in season has significant ($p < 0.01$) and site and season together has significant($p < 0.05$)(**Table 5**).

Dissolved reactive phosphorous (DRP*)* In autumn sample DRP ranged between 0.53 ppm in MGF and TEP to 0.01 ppm in DFF, in winter sample DRP ranged between0.49 ppm in DFF to 0.28 ppm in SOSF, in spring sample DRP ranged between 0.39 ppm in SOSF to 0.16 ppm in TEP, finally in summer sample DRP ranged between 2.79 ppm in SOSF to 0.47 ppm in MGF (Table 4) and Dissolved reactive phosphorous in season was significant ($p < 0.05$) **Table5**.

Nitrite (NO_2-N) In autumn sample NO_2-N ranged between 0.072 ppm in SOSF to 0.029 ppm in TEP, in winter sample NO_2-N ranged between 0.078 ppm in DFF to 0,026 ppm in SODF, in spring sample NO2-N ranged between 0.072 ppm in SOSF to 0.029 ppm in TEP, finally in summer sample NO_2-N ranged between 0.553 ppm in SOSF to 0.059 ppm in TEP and DFF with no significant as showing in (**Table 5**).

Nitrate In the ecosystem, nitrate is the most important nutrient. A higher value of Nitrate is due to water bodies polluted by organic matter. In the present investigation the values of concentrations of nitrates are ranged between 0.044 to 49.75 ppm (**Table 4**) with no significant as showing in (**Table 5**).

Ammonium (NH_4-N) in autumn sample NH_4-N ranged between 1. 64 ppm in SOSF to 0.92 ppm in TEP, in winter sample NH_4-N ranged between 1.56 ppm in DFF to 0.79 ppm in SOSF, in spring sample NH_4-N ranged between 1.45 ppm in SOSF to 0.58 ppm in TEP, finally in summer sample NH4-N ranged from 9.81 ppm in SOSF to 1.25 ppm in TEP and DFF (**Table 4**) with no significant as showing in (**Table 5**).From (Table 5) temperature has high significant $P \leq 0.001$ in site and site * season and $P \leq 0.01$ in season, DO has significant $P \leq 0.01$ in site and $P \leq 0.05$ in site * season, BOD has significant $P \leq 0.001$ in site and site * season, COD has significant $P \leq 0.001$ in season and $P \leq 0.05$ in site * season .DRP has significant $P \leq 0.05$ in season and All of Ca^{++},Mg^{++}, Na^{+}, K^{+}, $HCO3^{-}$,Cl-, SO, CO_3^{--}, NO_3,NO_2 and NH_4 has no significant (**Table5**).

Isolation of the resistant fungi: In the present study, 111 fungal isolates were isolated from water and sediment samples collected from the four investigated factories (Delta fertilizer factory (DFF), Talkha electric power plant (TEP), Marble and Granite factory (MGF) and Sandoup oil and soup factory) where heavy metals and other pollutants have been emitted in industrial effluents for several years.

As showing in (**Table 6**) 55 and 56 resistant fungal isolates resistant for Pb and Cr respectively were isolated from sediment and water of the four factories from the four seasons. For chromium SOSF recorded 10 isolates from water and 6 isolates from sediment, DFF recorded 5 isolates from water and 6 isolates from sediment,MGF recorded 8 isolates from water and 3 isolates from sediment and TEP recorded 11 isolates from water and 6 isolates from sediment, while for lead SOSF recorded 8 isolates 16 isolates 8 from water and 8 from sediment, DFF recorded 9 isolates from water and 6 isolates from sediment ,MGF recorded 11 isolates from water and 5 isolates from sediment and finally TEP recorded 7 isolates from water and 2 isolates from soil .

Table 4: Seasonal variation in physicochemical parameters of the effluent samples of the four investigated factories (Delta fertilizer factory (DFF), Talkha electric power plant (TEP), Marble and Granite factory (MGF) and Sandoup oil and soup factory)

Items	**Autumn**				**Winter**				**Spring**				**Summer**			
	MGF	**TEP**	**DFF**	**SOSF**	**MGF**	**TEP**	**DFF**	**SOSF**	**MGF**	**TEP**	**DFF**	**SOSF**	**MGF**	**TEP**	**DFF**	**SOSF**
Ca^{++}	1.73	1.59	2.33	7.95	2.80	1.84	2.89	1.09	2.91	1.35	2.33	3.19	2.77	0.82	0.82	8.85
Mg^{++}	1.05	1.19	1.87	5.32	1.98	1.12	2.05	0.81	2.05	0.87	1.41	1.92	1.19	0.65	0.65	6.21
Na^{+}	5.31	4.85	7.39	26.91	9.81	6.28	10.18	3.18	9.38	4.10	7.87	11.47	9.57	2.25	2.25	30.29
K^{+}	0.41	0.34	0.51	1.82	0.51	0.26	0.48	0.22	0.66	0.28	0.49	0.72	0.67	0.18	0.18	1.95
CO_3^{--}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HCO_3^{-}	1.88	1.65	2.47	8.19	2.96	6.63	3.01	1.20	3.12	1.47	2.51	3.35	2.86	0.98	0.98	9.14
Cl^{-}	4.98	4.12	6.79	24.36	8.93	5.72	8.85	2.98	8.06	3.92	7.09	10.12	8.97	1.93	1.93	28.14
SO_4	1.64	1.93	2.84	9.95	3.21	2.85	3.74	1.12	3.82	1.21	2.50	3.83	2.37	0.99	0.99	10.12
COD	7.61	6.14	8.22	9.75	27.2	23.1	29.6	16.8	29.7	18.9	27.4	33.8	36.5	17.9	45.1	59.8

DRP	0.53	0.53	0.01	0.49	0.42	0.37	0.49	0.28	0.35	0.16	0.28	0.39	0.85	0.47	1.35	2.79
NO_3-N	0.059	0.044	0.071	0.088	6.77	5.98	8.19	2.87	6.78	3.05	5.33	7.82	12.79	5.68	5.68	49.75
NO_2-N	0.062	0.029	0.050	0.072	0.063	0.050	0.078	0.026	0.062	0.029	0.050	0.072	0.135	0.059	0.059	0.553
NH_4-N	1.13	0.92	1.35	1.64	1.37	1.20	1.56	0.79	1.27	0.58	1.02	1.45	2.63	1.25	1.25	9.81

Where Ca^{++},Mg^{++}, Na^{+}, K^{+}, HCO_3^{-},Cl^{-}, $SO_4$and $CO_3^{--}$is represented by Meq / L and COD ,DRP ,NO_3,$NO_2$and NH_4 is represented by ppm

Table 5 showed the variation in P and F values among water temperature, PH , EC , DO , BOD Ca^{++},Mg^{++}, Na^{+}, K^{+}, $HCO3^{-}$,Cl^{-}, SO_4, CO_3^{--}, COD ,DRP ,NO_3,NO_2 and NH_4 in water in relation to variation to site and season .

Table 5: F and P values of variation of physicochemical parameters concentrations in water in relation to variation to site and season

variables	Site		season		Site*season	
	F value	**P value**	**F value**	**P value**	**F value**	**P value**
Temperature	34.310	0.000***	8.563	0.005**	21.43	0.000***
EC	2.716	0.107 ns	0.663	0.595 ns	1.690	0.230 ns
pH	1.943	0.193 ns	0.363	0.781 ns	1.153	0.407 ns
DO	7.542	0.008**	0.154	0.925 ns	3.848	0.035*
BOD	11.994	0.002**	1.097	0.399 ns	6.546	0.007**
Ca^{+2}	2.523	0.123 ns	0.341	0.797 ns	1.432	0.301 ns
Mg^{+2}	2.330	0.143 ns	0.336	0.800 ns	1.333	0.335 ns
Na^{+}	2.517	0.124 ns	0.271	0.845 ns	1.394	0.314 ns
K^{+}	3.141	0.080 ns	0.703	0.574 ns	1.922	0.182 ns
CO_3	.	.	.	.	.	.
HCO_3	1.202	0.363 ns	0.108	0.963 ns	0.655	0.688 ns
Cl	2.535	0.122 ns	0.296	0.827 ns	1.415	0.307 ns
SO_4	2.240	0.153 ns	0.234	0.870 ns	1.237	0.371 ns
COD	1.731	0.230 ns	8.610	0.005**	5.171	0.014*
DRP	0.978	0.445 ns	3.644	0.057*	2.311	0.125 ns
NO_3	0.929	0.465 ns	2.085	0.173 ns	1.507	0.276 ns
NO_2	1.107	0.396 ns	1.567	0.264 ns	1.337	0.334 ns
NH_4	1.157	0.378 ns	1.574	0.262 ns	1.366	0.323 ns

*** = Significant at $P \leq 0.05$; ** = Significant at $P \leq 0.01$, *** = Significant at $P \leq 0.001$ and ns= no significant**

Table 6: Number of resistant fungal isolate s for chromium and lead isolated from the four investigated factories (Delta fertilizer factory (DFF), Talkha electric power plant (TEP), Marble and Granite factory (MGF) and Sandoup oil and soup factory).

Number of heavy metal resistance isolates	Isolation sites								Total number of isolates
	SOSF		DFF		MGF		TEP		
	W	S	W	S	W	S	W	S	
Number of Chromium resistant fungal isolates	10	6	5	6	8	3	11	6	56
Number of Lead resistant fungal isolates	8	8	9	6	11	5	7	2	55

Where W is water, S is sediment.

Determination of minimum inhibitory concentrations (MICs): In this study the metal ions were added separately to PDA medium at concentrations of 400 and upto 7000 ppm .The plates were inoculated with 8 mm agar plugs from young fungal colonies, pre-grown on PDA and the linear growth was observed. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of metal that inhibit visible growth of the isolate [32].

At lower metal ions concentrations, the tested fungal isolates were very resistant and exhibited strong growth, higher metal ions concentration caused a reduction in growth a reduction in the growth rate is a typical response of fungi to toxicants [41]. Among 111 fungal isolates which isolated from the water and sediment sample of the 16 samples of the four investigated factories (Delta fertilizer factory (DFF), Talkha electric power plant (TEP), Marble and Granite factory (MGF) and Sandoup oil and soup factory) for the four seasons, *Aspergillus niger* (9isolates) , *Aspergillus flavus* (16 isolates) , *Aspergillus terreus* (9 isolates), *Penicillium purpurogenum* (4 isolates), *Cunninghamella sp* (7 isolates) , *Fusarium oxysporum* (4 isolates) , *Trichoderma viride* (14 isolates) , was the most frequent isolates ,so MIC is done to them to test their ability to resist Cr and Pb at different concentrations as shown in (**Table 7 and Table 8**).

Lead ions appeared to be less toxic in comparison with the others metals studied, for lead by increasing the concentration of lead ion; the diameter of growth of the fungal disk decreases as showed (in **Table 8**). *Aspergillus terreus* was the most resistant fungal isolate with MIC value of 6000 ppm, followed by *Aspergillus niger*, *Aspergillus flavus*, *Penicillium purpurogenum* and *Trichoderma viride* with MIC value of 5000 ppm. On the other hand *Cunninghamella sp. and Fusarium oxysporum.* showed the lowest MIC values of 4000 and 2000 ppm respectively , Ezzouhri *et al.* [19] found that all isolated strains were able to grow in plates amended with lead with MICs ranging from 20 to 25 mM, 7.5 - 25 mM and 12.5 - 15 mM for the genera *Aspergillus*, *Penicillium* and *Fusariumy*, respectively. Hexavalent chromium (Cr^{6+}) is the toxic form of chromium released during industrial processes such as leather tanning and pigment manufacture [42]. All isolates studied tolerated more than 7000 ppm of Cr as showed in **Table 8**.

Table 7: Minimal inhibitory concentrations (MICs) of fungal isolates for lead ion, fungal linear growth represented by (cm)

Fungal Species	**Lead ions concentrations (ppm)**								
	400	800	1000	2000	3000	4000	5000	6000	7000
Aspergillus niger	8.3	6.8	6.6	5.7	3.9	2.3	NG	NG	NG
Aspergillus flavus	6.8	6.7	6.6	4.3	1.8	1.65	NG	NG	NG
Aspergillus terreus	7.3	7.3	7.1	3.6	2.6	1.9	1.83	NG	NG
Penicillium purpurogenum	8.5	7.8	7.8	7.1	3.56	3.15	NG	NG	NG
Cunninghamella sp.	8.5	8.5	7.0	6.4	2.0	NG	NG	NG	NG
Fusarium oxysporum	8.5	7.9	5.9	NG	NG	NG	NG	NG	NG
Trichoderma viride	8.5	8.5	8.5	6.7	3.4	2.9	NG	NG	NG

The linear growth is measured by cm, NG= no growth

Table 8: Minimal inhibitory concentrations (MICs) of fungal isolates for chromium ion, fungal linear growth represented by (cm)

Fungal Species	**Chromium ions concentrations (ppm)**								
	400	**800**	**1000**	**2000**	**3000**	**4000**	**5000**	**6000**	**7000**
Aspergillus niger	8.5	8.5	8.5	8.5	8.5	8.5	5.5	7.5	7.0
Aspergillus flavus	8.5	8.5	7.5	7.0	6.3	6.5	6.0	5.8	5.68
Aspergillus terreus	8.5	8.5	7.0	5.8	5.2	3.85	3.1	3.1	2.8
Penicillium purpurogenum	8.5	8.5	8.5	8.0	7.5	6.75	6.0	5.3	3.5
Cunninghamella sp.	8.5	8.5	8.5	8.5	8.5	8.5	7.5	7.75	7.35
Fusarium oxysporum	8.5	8.5	8.5	8.5	8.5	8.5	7.3	7.0	5.0
Trichoderma viride	8.5	8.5	8.4	7.8	7.6	6.9	6.6	6.0	6.0

The linear growth is measured by cm, NG= no growth

For chromium all of isolates have MIC value higher than 7000 ppm as showing, *Cunnghamella sp* and *A.niger* followed by *Trichoderma viride*, *Aspergillus flavus* and fusarium *oxysporium* respectively show the highest rate of growth while *Aspergillus terreus* and *Penicillium purpurogenum* showed lower growth rate as shown in (table 9). Ezzouhri *et al.* [43] found that the most tolerant isolate belonged to the genus *Fusarium* with a MIC of 25 mM. *Penicillium* and *Aspergillus* isolates were also very tolerant to chromium (up to 10 and 15 mM, respectively).

Identification of the most potential isolates: All isolates were identified on the basis of their morphological characteristics (colonial morphology, color, texture, shape, diameter and appearance of colony.Among 111 the isolates of resistant fungi for Chromium and Lead which isolated from the water and sediment sample of the 16 samples of the four investigated factories (Delta fertilizer factory (DFF), Talkha electric power plant (TEP), Marble and Granite factory (MGF) and Sandoup oil and soup factory) for the four, several genera and several species which belong to Zygomycetes , Ascomycetes and Deutromycetes were isolated from the four factories water and sediment samples there are (*Cunninghamella*, *Aspergillus*, *Penicillium*, *Cephalosporium*, *Trichoderma* and *Fusarium*)seasonal variation between them are shown in (**Table 9**).

It is obvious from the results in (**Table 9**) that 111 fungal isolates belonging to three classes namely *Zygomycetes* , *Ascomycetes* and *Deutromycetes* were isolated from different investigated samples , *Ascomycetes* and *Deutromycetes* were the most predominant classes, *Ascomycetes* represented by 2 genera (*Aspergillus and Penicillium*) with 5 species (*Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Penicillium purpurogenum* and *Penicillium chrysogenum*) where *Deutromycetes* represented by 3 genera (*Cephalosporium* ,*Trichoderma* and *Fusarium*) with 4 species (*Cephalosporium sp* ,*Trichoderma viride* , *Trichoderma harzianum* and *Fusarium oxysporium*), while *Zygomycetes* represented by1 genera (*Cunninghamella*) with one species (*Cunninghamella sp*.) ,the summer samples recorded the greatest number of isolates among other seasons (39 isolates) followed by winter samples (26 isolates) followed by spring samples (25 isolates).

All seasons have species belong to the 3 classes while autumn samples recorded the lowest number of isolates (21 isolates), *Tricoderma harzianum* , *Trichoderma viride* and *Aspergillus flavus* was the most predominant isolates (22 isolates) ,(33 isolates) and (16 isolates) respectively from different isolated factories in different seasons ,the differences between the sampled sites regarding their richness on microbial isolates appear to be closely linked to the degree of heavy metal pollution. Generally, pollution of soil and water by heavy metals may lead to a decrease in microbial diversity as showing in (**Table 9**). Ezzouhri *et al.*[43] found that Fungi isolated belonged to the genera *Aspergillus*, *Penicillium*, ,*Alternaria*, *Geotrichum* and *Fusarium*..

Table 9: Seasonal variation in number of different fungal isolates for the four investigated factories (Delta fertilizer factory (DFF), Talkha electric power plant (TEP), Marble and Granite factory (MGF) and Sandoup oil and soup factory

Fungal classes	Autumn				Winter				Spring				Summer			
	SOSF	DFF	MGF	TEP	SOSF	DFF	MGF	TEP	SOSF	DFF	MGF	TEP	SOSF	DFF	MGF	TEP
Zygomycetes																
Cunninghamella sp.	0	1	0	0	0	2	0	0	1	0	0	1	1	0	1	0
Ascomycetes																
Aspergillus flavus	1	0	1	0	0	0	0	1	1	2	0	1	2	3	4	0
Aspergillus niger	0	1	0	0	3	1	1	0	0	0	0	0	0	2	0	1
Aspergillus terreus	0	0	0	0	0	0	0	0	0	0	0	0	3	2	1	3
Penicillium chrysogenum.	0	0	0	0	0	0	1	2	0	0	0	0	0	2	1	1
Penicillium purpurogenum	0	0	0	0	0	0	0	1	0	0	3	0	0	0	0	0
Deutromycetes																
Cephalosporium sp	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Trichoderma viride	1	1	2	6	5	0	3	2	3	1	1	2	1	3	0	2
Trichoderma harzianum	3	1	2	0	1	1	1	1	3	1	3	1	0	2	0	2
Fusarium oxysporium	0	1	0	0	0	0	0	0	0	0	1	0	2	0	0	0
Total number	**5**	**5**	**5**	**6**	**9**	**4**	**6**	**7**	**8**	**4**	**8**	**5**	**9**	**14**	**7**	**9**

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*** Corresponding author:** Gehad Fawzy;

Botany Department, Faculty of Science, Mansoura University, Egypt