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Studies on the mycoflora associated with sugarcane factory waste and pollution of River Nile in upper Egypt

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Sixty-nine species and four varieties which belong to twenty eight genera of terrestrial fungi were recovered from polluted and nonpolluted water and mud samples on glucose and cellulose-Czapek's agar at 28˚C. The most common species from the two substrates on the two types of media were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Cladosporium cladosporioides, Fusarium oxysporum, Mycosphaerella tassiana* and *Penicillium chrysogenum.* Twenty-six species belonging to 14 genera were isolated from polluted (26 species and 14 genera) and nonpolluted (17 and 10) mud samples on sabouraud's dextrose agar at 28°C. The most prevalent species were *Acremonium retiulum, Alternaria alternata, A. flavus, Aphanoascus fulvescens, Aspergillus terreus, Aphanoascus* sp., *Penicillium funiculosum* and *Stachybotrys chartarum.*

Key words: Pollution, River Nile, terrestrial fungi and keratinophilic fungi.

INTRODUCTION

Freshwater fungi including those of strictly aquatic and those of terrestrial habitats have been continuously studied for about one century and commonly found in pools, ponds, lakes, rivers, streams and bogs, as well as in marginal soils. They live as saprophytes or parasites on plants and animals. Many investigations were carried out on terrestrial fungi in the world (Park, 1974; El-Hissy, 1979; Moustafa and Khosrawi, 1982; Bettucci and Rodriguez, 1989; El-Hissy et al.,1989; 1990b; Bettucci et al., 1990; El-Nagdy and Abdel-Hafez, 1990; Moharrum et al., 1990; Rodriguez et al., 1990; Khallil and Abdel-Sater, 1992; Bettucci et al., 1993; Hyde and Goh, 1999; El-Hissy et al., 2001; Ho et al., 2003; Cai et al., 2002; 2003; 2006; Petra et al., 2005; Jiao et al., 2006; Mudur et al., 2006; Cai and Hyde, 2007; Jiang et al., 2008; Mongkol and Kevin, 2008). The present investigation is aimed to study the effect of sugarcane factory pollutants on the occurrence and distribution of terrestrial fungal population in the water and submerged mud of the River Nile.

The occurrence and distribution of keratinophilic and related fungi of different mud habitats have been investigated (Hassan, 1982; 1991; Mangiarotti and Caretta, 1984; Miyoshi et al., 1985; Hassan and Batko, 1986; Chabasse, 1988; Hassan and Shoulkamy, 1991; Soon, 1991; Abdullah and Dina, 1995; Abdullah and Hassan, 1995; Ulfig et al., 1996; 1997; Ali-Shtayeh et al., 1999; Ali-Shtayeh and Rana, 2000).

MATERIALS AND METHODS

Thirty samples of polluted and nonpolluted water and submerged mud were collected from River Nile during the working season of Nag Hamady sugarcane factory from December - April 2007 - 2008. The water samples were analyzed chemically for the estimation of total soluble salts and organic matter contents (Jackson, 1958). A pH-meter was used for pH determination of water and submerged mud.

Determination of terrestrial fungi

The dilution plate method (Johnson and Curl, 1972) was used for the estimation of fungi in submerged mud samples. For the recovery of

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terrestrial fungi from the aggregated water, 1 ml of each water sample was transferred into each of five petri-dishes. A Modified Czapek's agar medium in which glucose (10 g/L) or powder cellulose (20 g/L) were used for isolation of glucophilic and cellulose-decomposing fungi, respectively. Streptomycin (20 u/ml) and Rose Bengal (30 ppm) were added as bacteriostatics agents. The plates were incubated at 28°C for 7 days and the developing fungi were counted and identified (Morphologically, based on macro- and microscopic characteristics). The fungal numbers were calculated per 1 ml water in every sample.

Determination of keratinophilic and related fungi

The dilution plate method (Johnson and Curl, 1972) was used for the estimation of fungi in mud samples on Sabouraud's dextrose agar medium (Moss and McQuown, 1969), which was supplemented with chloramphenicol (0.5 mg / ml medium) and cycloheximide (0.5 mg / ml medium). The plates were incubated at 28°C for 4 to 6 weeks and the developing fungi were counted and identified.

RESULTS AND DISCUSSION

The total soluble salts of polluted water and submerged mud samples ranged between 0.1 to 1.9 and 1 to 9% and their contents of organic matter fluctuated between 0.003 to 0.58 and 2.3 to 51.9%, respectively. El-Hissy et al. (2001) found that the total soluble salts and organic matter contents in water and submerged mud of the River Nile polluted with Kom Ombo sugar cane factory fluctuated between 125 to 540 mg/L, 0.262 - 0.678% and 0.34 to 6.54 mg/L, 0.88 to 2.85%, respectively. The pH values of the water and submerged mud samples were alkaline and ranged between 7.3 to 8.8 and 8.5 to 9.8, respectively. These results are in agreement with the results of El-Hissy et al. (2001) who found that pH ranged between 5.4 to 8.19 and 8.05 to 8.6, respectively, in a similar context using similar methods. Also, the total soluble salts of nonpolluted water and submerged mud samples tested fluctuated between 0.05 to1.5 and 1 to 7% and their contents of organic matter ranged between 0.003 to 028 and 2.3 to 43.3%, respectively. The pH values of the water and submerged mud samples ranged between 7.6 to 9.1 and 8.8 to 10.9, respectively.

Polluted terrestrial fungi

Sixty-five species and four varieties belonging to 28 genera were isolated from water (21 genera and 49 species plus 3 varieties) and submerged mud (25 genera and 57 species plus 2 varieties) samples on glucose- and cellulose-Czapek's agar at 28°C (Table 1). In this respect, El-Hissy et al. (2001) isolated 41 species which belong to 32 genera of terrestrial fungi from water and mud samples polluted by industrial effluents of Kom Ombo sugar cane factory on glucose-and cellulose-Czapek's agar at 28°C. The most common genera from the two substrates on the two types of media were *Acremonium, Aspergillus, Cladosporium, Fusarium, Gibberella, Mucor, Mycosphaerella, Penicillium* and *Trichoderma*. They occurred in 40 to 100, 26.7 to 93.3, 33.3 to 100 and 26.7 to 100% of the samples comprising 1.4 to 32.9, 2.9 to 20.5, 1.7 to 38.7 and 0.5 to 46.9% of total fungi, respectively. From the above genera the most prevalent species were *Acremonium strictum, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Cladosporium cladosporioides, Cladosporium sphaerospermum, Fusarium oxysporum, Gibberella fujikurio, Mucor circinelloides, Mycosphaerella tassiana, Penicillium chrysogenum, Penicillium oxalicum, Penicillium puberulum* and *Trichoderma harzianum*. They were encountered in 13.3 to 93.3, 26.7 to 93.3, 26.7 to 100 and 26.7 to 100% of the samples comprising 0.2 to 17.5, 0.7 to 12.8, 0.7 to 20.2 and 0.3 to 24% of total fungi, respectively (Table 1). Ali-Shtayeh and Rana (2000) found that the most common fungi in polluted field soils and raw city sewage in Jordan were *Alternaria alternate, Aspergillus candidus, Geotrichum candidium* and *Paecilomyces lilacinus*.

Most of the above species were encountered previously but with various numbers and frequencies from water and mud of Ibrahim canal (Abdel-Hafez and Bagy, 1985), River Nile (El-Hissy et al., 1990a), Aswan High Dam Lake (El-Hissy et al., 1990b), some ponds of Kharga Oases (El-Nagdy and Abdel-Hafez, 1990), water, soil and air polluted by the Manquabad Superphosphate (Khallil and Abdel-Sater, 1992), and River Nile polluted with industrial effluents of Kom Ombo sugar cane factory (El-Hissy et al., 2001) in Egypt. Also, the previous fungal species were reported from the world (Barlocher and Kendrick, 1974; El-Hissy, 1979; Hiremath et al., 1985; Bettcci and Roquebert, 1989; Bettucci et al., 1990; 1993; Hyde and Goh, 1999; El-Hissy et al., 2001; Cai et al., 2002; 2003; 2006; Ho et al., 2003; Petra et al., 2005; Jiao et al., 2006; Mudur et al*.,* 2006; Cai and Hyde, 2007; Jiang et al., 2008; Mongkol and Kevin, 2008). The remaining genera and species were moderate or less frequent (Table 1).

Nonpolluted terrestrial fungi

Forty-six species and 2 varieties in addition to 20 genera were collected from water (10 genera and 29 species) and submerged mud (18 and 39 plus 2 varieties) samples on glucose and cellulose-Czapek's agar at 28°C (Table 1). The most prevalent genera from the two substrates on the two types of media were *Aspergillus, Cladosporium, Fusarium, Mycosphaerella, Penicillium* and *Trichoderma*. They were found in 26.7 to 93.3, 26.7 to 86.7, 26.7 to 86.7 and 26.7 to 86.7% of the samples constituting 1.6 to 37.7, 1.2 to 58.7, 2.1 to 37.7 and 1.02 to 37.1% of total fungi, respectively. From the above genera the most common species were *A. flavus, A. fumigatus, A. niger, C. cladosporioides, F. oxysporum, Penicillium chrysogenum* and *Penicillium corylophilum.*

Table 1: Total counts (TC, calculated per g in all samples) number of cases of isolation (NCI, out of 15) and occurrence remarks (OR) of fungal genera and species recovered from polluted and nonpolluted water and mud on glucose and cellulose–Czapek's agar at 28°C.

Genera and species	Polluted								Nonpolluted							
	Water				Mud				Water				Mud			
	Glucose		Cellulose		Glucose		Cellulose		Glucose		Cellulose		Glucose		Cellulose	
	TC	NCI and OR	TC	NCI and OR	TC	NCI and OR	TC	NCI and OR	TC	NCI and OR	TC	NC and OR	TC	NCI and OR	TC	NCI and OR
Acremonium	2750	6M	1200	5M	4250	9H	2050	5M	250	2L			150	1R	700	5M
A.cerealis (Karst.)W.Gams	450	4M			500	3L	350	2L	100	1R					50	1R
A.furcatum F.& V. Moreau							150	1R	50	1R						
A.murorum (Corda) W.Gams	100	1R			250	2L										
A.retiulum W.Gams	1400	3L			1700	3L	600	2L							100	1R
A.strictum W.Gams	800	6M	1200	5M	1800	8H	950	4M	100	2L			150	1R	550	5M
Alternaria					600	4M	1850	7M			50	1R	2050	8H	3400	7M
A.alternata (Fries) Keissler					600	4M	1850	7M			50	1R	1750	8H	3000	7M
A.chlamydospora Mouchacca													150	1R	100	1R
A. tenuissima (Kunze:Pers.) Wiltshire													150	1R	300	1R
Aspergillus	20050	15H	8250	14H	33850	15H	34450	15H	9050	14H	15250	13H	18600	13H	18200	13H
A.candidus Link	50	1R			200	2L			950	1R			800	3L		
A.carneus (V.Tiegh.)Blochwitz	150	2L			150	2L							400	3L		
A.flavus Link	10950	14H	1100	14H	13800	15H	17650	15H	4700	14H	9400	13H	8450	13H	9750	12H
A. flavus var. *colmnaris* Rapper and Fennell																
A.fumigatus Fresenius	3450	13H	4150	12H	7300	15H	4800	13H	2050	10H	2150	10H	4000	12H	3750	12H
A.niger Van Tieghem	2850	13H	2200	9H	4550	12H	2300	13H	800	8H	2850	8H	1850	10H	3000	8H
A. ochraceus Wilhelm	150	2L	350	3L	650	5M			50	1R			100	1R	400	4M
A. sydowii (Bain and Sart.) Thom and Church					2250	8H	250	2L					1600	5M	450	1R
A. terreus Thom	350	3L			0600	7M	6650	8H	500	5M	850	2L	1200	6M	850	2L
A. terreus var aureus Thom and Raper	200	2L	450	2L	2300	7M	2800	6M					200	4M		

Table 1. Contd.

A.ustus Fennell and Raper					550	5M										
A.versicolor (Vuill.)Tiraboschi	100	1R			500	4M										
Botryotrichum atrogriseum Van Beyma							100	1R							100	2L
Chaetomium							1050	3L								
C.globosum Kunze							800	3L								
C.spirales Zoph							250	1R								
Cladosporium	1550	7M	1200	4M	7050	11H	2700	9H	1750	7M	500	4M	5000	8H	1850	4M
C.cladosporioides (Fres.)de Vries	1450	6M	900	4M	5100	11H	1950	9H	1550	7M	500	4M	2400	8H	1850	4M
C.sphaerospermum Penzig	100	2L	300	4M	1950	5M	750	5M	200	2L			2600	4M		
Cochliobolus spicifer Nelson	100	1R														
Emericella nidulans (Edidam)Vuillemin	100	1R					100	1R	100	1R	150	2L				
Epicoccum nigrum Link	100	1R			100	1R										
Fenniellia flavipes Wiley and Simmons	200	1R											500	2L		
Fusarium	5250	13H	5250	9H	5100	7M	4500	13H	400	4M	300	4M	2300	6M	5450	8H
F.moniliforme Sheldon			1250	7M												
F.oxysporum Shelecht	4400	13H	3800	9H	3100	7M	4400	13H	300	4M	300	4M	650	4M	3650	8H
F.poae (Peck) Wollenweber	200	2L			200	2L										
F.semitectum Berk.& Rav.	400	3L	200	2L	1100	6M			50	1R			1650	3L	1800	2L
F.tricinctum (Corda) Sacc.	250	2L			700	3L	100	1R	50	1R						
Gibberella	850	7H	4400	8H	1500	5M	800	5M	100	2L	250	2L	300	2L	150	1R
G.acuminata Wollenweber	200	3L			100	1R										
G.avenacea Cooke	100	2L														
G.fujikuroi (Sawada) Ito	500	5M	4400	8H	1300	5M	800	5M			150	1R	150	2L	150	
G.intricans Wollenweber					100	1R					100	1R	150	2L		
G.zeae (Schwabe)Petch	50	1R														
Humicola grisea (Tassi) Goid					200	3L	1250	4M								
Mucor	2200	6M	1950	4M	1900	7M	600	4M					850	5M		
M.circinelloides Van Tieghton	1050	5M	600	5M	1050	6M	400	4M					600	5M		

Table 1. Contd.

M.racemosus Fresenius	1150	4M	1350	3L	850	7M	200	2L					250	3L	6300	
Mycosphaerella tassiana (Albertini and Schweinti)	2500	8H	1400	4M	7600	13H	400	4M	2650	11H	450	8M	8600	11H		
Mycothecium verrucaria (Alb. and Sch.) Dit.							100	1R			100	1R				
Nectria	2100	8H	2300	9H	1500	4M	7600	11H					400	3L		
N.haematococca Berkeley and Brown	2100	8H	2300	9H	1400	4M	7500	11H					400	3L		
N.viridescens C.Booth					100	1R	100	1R								
Paecilomyces variotii Bainier							100	1R					100	1R		
Penicillium	20150	13H	9050	12H	19400	15H	12350	12H	9550	10H	7450	9H	8750	13H	9900	12H
P.aurantiogriseum Dierckx					100	1R							100			
P.brevicompacium Dierckx	100	2L			150	2L							100	1R		
P.chrysogenum Thom	10800	13H	5150	12H	4750	13H	7200	12H	2600	9H	5150	9H	3650	11H	6650	12H
P.citrinum Thom	1150	7M	750	5M	1400	8H			650	5M			700	5M	200	3L
P.coryloohilum Dierckx	700	7M	350	1R	3900	7M			1950	5M	1950	6M	850	5M	400	4M
P.duclauxii Delacroix	500								50	1R						
P.funiculosum Thom					300	2L			3500	7M						
P.oxalicum Currie andThom	2150	8H	1100	4M	6600	6M	2900	4M	800	2L	100	2L	900	4M	750	2L
P.purberulum Bainier	4700	12H	1700	7M	2200	8H	2250	5M			250	5M	2450	5M	1900	7M
P.viridicatum Westling	50	1R														
Phoma glomerata (Corda) Woolenweber and Hochapfel	900	3L	1350	4M	50	1R	450	2L	700	2L			100	1R	900	3L
Rhizopus stolonifer (Ehrenb.)Link	50	1R			50	1R	400	3L								
Scopulariopsis brevicaulis (Sacc.)Bainier			100	1R	200	2L										
Scytalidium lignicola Pesante. *Stachybotrys*			150	1R	950	3L	400	2L					500	4M	1400	2L
S.atra var. *microspora* Mathur and Sankhla							100	1R							1350	2L

Table 1. Contd.

S. chartarum (Ehrenb: Lindt) Hughes					850	3L	300	2L					500	4M	50	1R
S.cylindrospora C.W.Jensen					100	1R										
Torula herbarum (Pers.) Link					50	1R	250	1R					50	1R		
Trichoderma	2200	9H	3550	8H	2200	6M	1650	6M	750	5M	1500	8H	1050	4M	500	4M
T..hamatum (Bonord.)Bain	350	3L			1600	6M			50	1R						
T.harzianum Rafai	1700	9H	550	4M	600	4M	350	4M	150	3L	250	3L	550	4M	500	4M
T.pseudokoingii	150	1R	500	4M												
Rafai *T.viride* Pers			2500	8H			1300	6M	550	5M	1250	8H	500	3L		
Trichothecium roseum (Pers.) Link: Gary	50	1R			700	3L										
Trimmatostroma salicis Corda	150	1R			150	1R	350	2L								
Total counts	61250		40250		87400		73500		25300		26000		49300		49000	49000
Number of genera : 28	21				25				10				18			
Number of species : 69 + 4 Varieties	49 +3 varieties				57 +2 varieties				29							

They emerged in 26.7 to 93.3, 26.7 to 86.7, 26.7 to 86.7 and 26.7 to 80% of the samples comprising 1.2 to 18.6, 1.2 to 36.2, 1.3 to 17.1 and 0.8 to 19.9% of total fungi, respectively (Table 1).

Ali-Shtayeh and Rana (2000) found that the most common fungi in non-polluted field soils and raw city sewage in Jordan were *A. alternate, A. candidus, G. candidium* and *P. lilacinus*. All the above fungi were isolated from all over the world by several researchers (Barlocher and Kendrick, 1974; Abdel-Hafez and Bagy, 1985; Hiremath et al*.,* 1985; Bettucci et al., 1990; 1993; El-Hissy et al*.,* 1990a; b; El-Nagdy and Abdel-Hafez, 1990; Khallil and Abdel-Sater, 1992; Bettcci and Roquebert, 1995; Hyde and Goh, 1999; El-Hissy et al., 2001; Cai et al., 2002, 2003; 2006; Ho et al., 2003; Petra et al., 2005; Jiao et al., 2006; Mudur et al., 2006; Cai and Hyde, 2007; Jiang et al., 2008; Mongkol and Kevin, 2008). The remaining fungal genera and species were less common (Table 1). Numerous species were isolated only from polluted water and submerged mud on glucose or cellulose agar medium: *Acremonium mucorum*, *Aspergillus ustus*, *Aspergillus versicolor*, *Botryotrichum atrogriseum*, *Chaetomium spirales*, *Cochliobolus spicifer*, *Epicoccum nigrum*, *Fusarium poea*, *Gibberella acuminata*, *Gibberella avenacea*, *Humicola grisea*, *Nectria viridescens*, *Penicillium funiculosum*, *Penicillium viridicatum*, *Rhizopus stoloifer*, *Scopulariopsis brevicaulis*, *Scytalidium lignicola*, *Stachybotrys cylindrospora*, *Trichoderma pseudokoningii*, *Trichothecium roseum* and *Trimmatostroma salicis* (Table 1).

Keratinophilic and related fungi

Twenty-six species belonging to 14 genera were isolated from polluted (26 genera and 15 species) and nonpolluted (17 genera and 11 species) mud samples on sabouraud's dextrose agar at 28°C (Table 2). The most common genera were *Acremonium, Alternaria, Aspergillus, Aphanoascus, Penicillium* and *Stachybotrys* (Table 2). They occurred in 26.7 to 100 and 26.7 to 80% of the samples comprising 1.9 - 46.3 and 2.4 to 35.8% of total fungi, respectively (Table 2). From the above genera the most prevalent species were *Acremonium retiulum, A. alternate, A. flavus, Aphanoascus fulvescens, A. terreus, Aphanoascus* sp., *C. cladosporioides*, *P. funiculosum* and *Stachybotrys chartarum*. They

Table 2. Total counts (TC, calculated per g mud in all samples), number of cases of isolation (NCI, out of 15) and occurrence remarks (OR) of fungal genera and species recovered from polluted and nonpolluted mud samples on Sabouraud's dextrose agar at 28°C.

Genera and Species	Polluted mud		Nonpolluted mud	
	TC	NCI&OR	TC	NCI&OR
Acremonium	4800	10H	2550	7M
A. cerealis	200	2L	--	--
A. retiulum	2600	6M	2550	7M
A. strictum	2000	6M	--	--
Alternaria alternata	550	4M	1000	4M
phanoascus A	13600	15H	6050	12H
A. fulvescens	10700	13H	3600	9H
A. terreus	1000	6M	600	5M
Aphanoascus sp.	1900	8H	1850	6M
Aphinisia queenslandica	150	1R	300	3L
Aspergillus	2000	8H	2200	8H
A. flavus	500	4M	900	7M
A. fumigatus	300	2L	400	3L
A.sydowii	800	6M	--	--
A. terreus	300	1R	--	--
A.ustus	100	1R	900	5M
Chrysosporium	500	3L	300	1R
C. luteum	150	1R	150	1R
C. pannorum	350	3L	150	1R
Cladosporium	1400	4M	850	6M
C. cladosporioides	1200	4M	850	6M
C. sphaerospermum	200	2L	--	--
Epicoccum nigrum	100	1R	--	--
Fusarium oxysporum	100	1R	--	--
Humicola grisea	500	1R	--	--
Mycosphaerella tassiana	1500	6M	550	3L
Nectria haematococca	100	1R	--	--
Penicillium	3100	7H	2700	9H
P. chrysogenum	300	3L	550	4M
P. corylophilum	1400	5M	300	3L
P. funiculosum	1400	5M	1850	10H
Stachybotrys chartarum	1000	5M	400	4M
Total count	8450		16900	
Number of genera 14	14		10	
Number of species 28	28		18	

recovered from 26.7 to 86.7 and 26.7 to 66.7% of the samples contributing 1.7 - 36.4 and 2.4 - 21.3% of total fungi, respectively (Table 2). Ali-Shtayeh and Rana (2000) found that the most common fungi in polluted and non-polluted field soils and raw city sewage in Jordan were *Microsporum gypseum*, *Trichophyton ajelloi*, *Arthroderma cuniculi*, *Arthroderma curreyi*, *Chrysosporium keratinophilum*, *Chrysosporium tropicum* and *pannorum*. Most of the above species were isolated previously, but with various numbers and frequencies from the world (Garg, 1966; Ajello and Padhye, 1974; McAleer, 1980; Hassan, 1982; 1991; Cano et al., 1985; Hassan and Batko, 1986; Ogbonna and Pugh, 1987; Chabasse, 1988; Ulfig and Ulfig, 1990; Hassan and Shoulkamy, 1991; Soon, 1991; Abdullah and Dina, 1995; Abdullah and Hassan, 1995; Ulfig et al., 1996; 1997; Ali-Shtayeh et al., 1999).

Several authors have suggested that *A. fuluvescens* may be an opportunistic dermatophyte (Rippon et al., 1970; Marin and Campos, 1984), while Cano et al. (1990)

demonstrated that *A. fuluvescens* behaves as an internal opportunistic pathogen. Some species were isolated only from polluted mud such as: *Acremonium cerealis, Aspergillus strictum, Aspergillus sydowii, Aspergillus terreus, C. sphaerospermum, Epicoccum nigrum, F. oxysporum, Humicola grisea* and *Nectria haematococca.* The remaining genera and species were isolated in less frequents (Table 2). Some species were isolated only from polluted mud such as: *A. cerealis, A. strictum, A. sydowii, A. terreus, C. sphaerospermum, E. nigrum, F. oxysporum, H. grisea* and *N. haematococca.*

In conclusion, in this investigation the authors studied the effect of the pollutants of the Nag Hamady sugar cane factory on occurrence and distribution of aquatic and terrestrial fungal population in the water and submerged mud of the River Nile. This study shows that the numbers and frequencies of fungi in polluted water and submerged mud samples were higher than nonpolluted samples of water and submerged mud. This is due to the effect of wastes of the sugar cane factory in the River Nile, so pouring industrial factory wastes into River Nile must be avoided.

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