OnLine Journal of Biological Sciences 1 (11): 1046-1050, 2001

Environmental Impact of Sewage Water on Soil Variables, *Ricinus communis* and *Helianthus annuus* Protein and Associated Species

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Abstract: Two different provinces (El Khanka -Kalubya governorate in lower Egypt -'sewage water irrigation' and Abu Reish -Aswan governorate in upper Egypt - 'River Nile irrigation') were studied to compare their soil physical and chemical properties, accompanied weed species to *Ricinus communis* and *Helianthus annuus* and analysis of seed storage protein for both *R. communis* and *H. annuus*. Variable levels of: soil properties, associated weed species and storage seed protein banding patterns for both *R. communis* and *H. annuus* were identified. Accordingly, an environmental pollutants in El Khanka rather than Abu Reish has been concluded.

Key words: Sewage water, *R. communis, H. annuus,* soil properties, Egypt

Introduction

Increasing demand for the limited water supplies in Arid and Semi-Arid countries leads to the use of treated sewage water for agricultural activities. In Egypt sewage water is usually applied to sandy soils which show lack of organic matter as well as micro- and macro-nutrient elements. El-khanka (Kalubya governorate) is an example where treated sewage water is used for irrigation in Great Cairo area.

In recent years, agronomists and environmentalists became aware of the potential hazard attendant with leaching of heavy metals from sewage water for both (Eid, 1984; Volnik *et al.*, 1985; Porcel *et al.*, 1995) and growing-plants (Hinesly *et al.*, 1979; Volnik *et al.*, 1983; Brams and Anthony, 1988; Vandriel *et al.*, 1992; Dowdy and Nater, 1995). Further studies concerned with change of soil physical (Mansour, 1996) and chemical properties (Vlamis *et al.*, 1978) and effect of sewage water application on growing plants (Kirkham, 1986; Feigin *et al.*, 1991; Davies *et al.*, 1993; Liberti and Notarnicola, 1998; Quist *et al.*, 1999; Gori *et al.*, 2000) as well as changing biotic floral species (Shaheen, 1987; Hassan and Badri, 1995 and Saavedra *et al.*, 1990).

Weeds represent a highly specific and biologically important component of their environments. Their persistence is remarkable in view of efforts to eliminate them, and warrants greater attention (Radosevich and Holt, 1984). Estimation of crop production looses during 1991 due to weeds averaged $ 5984 million in Canada (Swanton *et al.*, 1993).

An increased understanding in the biology, ecology and genetics of weeds is needed to optimize their management. Research on weed control with biological agents and natural-products should be conducted with emphasis on optimizing performance in the field environments (Hess, 1994).

Many studies dealing with the weed flora of Egypt were concerned with the description on the floristic composition of chronological analysis rather than considering the relations of weed communities with other environmental variables and agricultural practices.

Weed floras show very marked differences depending on whether they are in cultivated or uncultivated areas. Weed communities must be studied in order to estimate changes in the flora exposed to mechanical and herbicidal controls (Montegut, 1974). Furthermore, the composition of weeds under different environmental and agricultural conditions must also be studied to improve the method for their control (Barralis and Chadoeuf, 1980).

This study, the physical and chemical soil analysis in two provinces (El Khanka "Kalubya governorate" and Abu Reish "Aswan governorate") in Egypt were carried out, screening the associated weed species where *R. communis* and *H. annuus* were grown. In addition, gene toxicity of applying sewage water was carried out using SDS-PAGE protein banding pattern for *R. communis* and *H. annuus* seeds.

Materials and Methods

Weed assemblages with two oil plants (*Ricinus communis* and *Helianthus annuus*) are recorded at Aswan during June 2000 and Qalubya during August 2000. Identification of weed flora was carried out according to Täckholm (1974), El-Hadidi and Fayed (1994/1995) and Boulos (1995). In addition, soil samples were collected from the two provinces and seed samples of both *R. communis* and *H. annuus*.

Soil samples were screened through 2 mm sieve and gravel was discarded. Different soil sizes of particles were separated using the pipette method (Kilmer & Alexander, 1949). The percentages of sand, silt and clay were calculated. Soil water extracts (1:5) were prepared for EC and pH determination using conductivity and pH meters, chlorides by direct titration against silver nitrate using potassium chromate as an indicator, carbonates and bicarbonates by direct titration against HCl using phenolphthalein and methyl orange as indicators. Calcium and magnesium were determined by titration against EDTA (ethylene-diamine dihydrogen tetracetic acid) using ammonium purpurate and Eriochromo black T. as indicators (Jackson, 1962).

Seeds of *R. communis* and *H. annuus* were collected for protein analysis. The applied protocol for seed storage protein banding pattern was followed according to Laemmli (1970). *R. communis* and *H. annuus* seeds of each sample were decoated and the cotyledons mild to fine powder. Total proteins were extracted overnight using 0.2 M Tris-HCl buffer, pH 3.8 containing SDS. After centrifugation at 9000 rpm for 6 minutes, the supernatant was collected. SDS slab gel of 12.5% acryl amide was used. Molecular weights of the seed storage protein banding patterns of each sample were identified using gel-pro analyzer V 3.O computer program.

Results and Discussion

Results of soil analysis for samples collected from Northern province in Middle Delta (Qalubya "El Khanka") and Upper Egypt (Aswan "Abu Riesh") under *Ricinus communis* and *Helianthus annuus* are shown in Table 1. The texture of the soil from Abu Riesh (sand=51.16, silt=15.66 and clay=24.99%) is finer than the soil from El Khanka (sand=87.78, silt=0.63 and clay=9.69%). The organic matter percentage at Abu Riesh (7.85±1.16) is higher than El Khanka (1.26±0.84). EC of the soil from El-Khanka (707±279 mmoh/cm) is higher than that of Abu Riesh (281.5±55.9 mmoh/ cm). Chlorides and calcium of the soil from El Khanka (15.96 ±4.13 and 22.89±17.56 mg/100gm) are higher than that of Abu Riesh respectively (7.27±1.75 and 13.63±1.41 mg/100gm) while HCO_3 content is higher for Abu Reish (33.26±2.17 mg/100gm) than from El Khanka (24.56±1.79 mg/gm). pH and magnesium are relatively the same values in both habitats with average (7.64±0.15 and 7.71±0.29) and (5.96±1.03 and 6.12±3.15 mg/100g) respectively.

The relatively high level of organic matter of Abu Riesh may be attributed to the effect of decomposition of remnants of previous plant growth. The high level of chlorides and calcium content at El Khanka may be attributed by the irrigation by sewage water. The high level of finer materials (alluvial materials) at Abu Riesh may be attributed to the deposition of fine materials during the flooding periods, while low level of the finer materials in El Khanka may be attributed to the effect of land reclamation and weathering processes.

Fifty weed species belonging to 21 families were recorded in El Khanka province associated to *Ricinus communis* and *Helianthus annuus*. The most represented families are Graminae (30%), Amaranthaceae (8%), Chenopodiaceae, Convolvulaceae, Cruciferae, Cyperaceae and Malvaceae (6%) and Compositae and Solanaceae (4%). Therophytes represent 56% of life-form spectrum while perennials formed 44%. This data is in agreement with Hassib (1951). On the other hand, forty-six weed species belonging to 18 families were recorded in Abu Riesh at Aswan province associated to *R. communis* and *H. annuus*. The most represented families were Graminae (32.6%), Leguminosae (10.86%), Compositae (10.86%), Convolvolaceae (6.52%) and Euphorbiaceae (6.52%). Therophytes represented 60.9% of the life-form spectrum while perennials form 39.1%. According to Egyptian flora (Hassib, 1951), therophytes (annuals) are the most common life form. Two species were recorded for the first time, *Celosia argentea* and *Vossia cuspidata* in Qalubya and Aswan respectively (Shaheen, 1987).

Chronological analysis of weed species collected during summer season and associated with *R. communis* and *H. annuus* in El Khanka and Abu Riesh provinces (Tables 2 and 3). Higher percentage of Mediterranean taxa in El Khanka (12%) than in Abu Riesh (6.5%) were observed.

Table 1: Weed species recorded in the different provinces (Aswan & Qalubya), A: annual; P: perennial, FC: Floristic categories: COMS=Cosmopolitan, PAN=Pantropical, PAL: Palaeotropical, S-Z=Sudano-Zambezian, SA-SI=Saharo-Sindian, IR-Tr=Iran-Turanian and ME=Mediterranean.

Species	Life form	Floristic category	Aswan	Qalubya
Abultilon pannosum	P	SA-SI+SZ	*	
Acacia nilotica	P	S-Z	*	
Acacia seyla	P	S-Z	*	
Alhagi graecorum	P	PAN	*	
Amaranthus bilotoides	A	PAN		*
Amaranthus hybrids	A	PAN		*
Amaranthus lividus	A	PAL	*	*
Argemon mexicana	A	PAN	*	
Arundo donax	P	ME+IR-TR		*
Beta vulgaris	A	ME+IR-TR		*
Brachraria eruciformis	A	PAL	*	
Brassica oleracea	A	ME		*
Cajanus cajan	A	PAL	*	
Calotropis procera	P	SA-SI+S-Z	*	
Celosia argentea	A	S-Z		*
Chenopodium album	A	PAN	*	
Chenopodium ambrosoides	A	PAN		*
Chenopodium murale	A	COSM		*
Cichorium endivia	A	ME	*	
Convolvulus arvenis	P	PAN	*	*
Conyza bonariensis	A	COSM	*	
Corchorus olitorius	A	PAL	*	*
Cynanchum acutum	P	ME+IR-TR		*
Cynodon dactylon	P	PAN	*	*
Cyperus alopecuroides	P	PAN		*
Cyperus articulatus	P	PAL		*
Cyperus rotundus	P	PAN	*	*
Cuscuta pedicellata	A	SA-SI+S-Z	*	*
Dactyloctenium aegyptium	A	PAN	*	*
Desmostachya dipinnata	P	SA-SI+S-Z +ME+IR-TR	*	
Dichanthium annulatum	P	PAL	*	
Digitaria sangunalis	A	PAN	*	*
Dinerbra retroflexa	A	PAL		*
Echinochlora colona	A	PAN	*	*
Echinochloa crusgalli	A	PAN		*
Eclita alba	A	PAN		*
Eragrostis cilianensis	A	PAN	*	
Eruca sativa	A	SA-SI		*
Euphorbia heterophylla	A	PAN	*	
Euphorbia hirta	A	S-Z	*	
Euphorbia peplis	A	PAN	*	
Gynandropsis gynandra	A	PAN		*
Hibiscus trionum	A	PAN		
Hyphaene thebaica	P	S-Z	*	
Imperata cylindrica	P	PAN		*
Impomea carica	P	PAL		*
Impomea eriocarpa	A	PAL	*	
Jussiaea repens	P	PAL		*
Lemna gibba	P	PAN		*
Leptochloa fusca	P	PAN		*
Malva parviflora	A	PAN		*
Mentha longifolia	P	ME+IR-TR+S-Z		*
Panium repends	P	COMS		*
Paspalidium geminatum	P	PAN	*	*
Paspalum distichum	P	PAN		*
Phoenix dactylifera	P	SA-SI+S-Z	*	*
Phramites australis	P	PAN	*	*
Pluchea dioscoridis	P	SA-SI+S-Z		*
Polgonum equisetiformae	P	PAN	*	
Polygonum monspliensis	A	COSM	*	
Portulaca oleracea	A	PAL	*	*

Pulicaria crispa	P	SA-SI+S-Z	*	
Raphanus sativus	A	COSM		*
Rumex dentatus	A	PAN		*
Senna occidentalis	P	PAL		*
Sesbaia sesban	P	PAL	*	
Setaria verticillata	P	ME		*
Setaria viridis	P	ME	*	
Sida alba	P	PAL	*	*
Solanum lycoperscum	P	COSM		*
Solanum nigrum	P	COSM	*	*
Sonchus oleraceaus	P	COSM	*	
Sorghum halepense	P	PAN	*	*
Trianthema portulacastrum	P	PAN	*	
Tribulus terrestris	P	PAN		
Typha domingensis	P	PAN		*
Vossia cuspidata	P	PAL	*	*
Xanthium strumarium	A	PAN	*	
Zea mays	A	COSM	*	

Table 2: The floristic categories and the life forms of the recorded species in the two studied areas (Aswan & Qalubya). A: annual; P: perennial, FC: Floristic categories: COSM=Compolitan, PAN=Pantropical, PAL: Palaeotropical, S-Z=Sudano-Zambezian, SA-SI=Saharo-Sindian, IR-TR=Irano-Turanian and ME: Mediterranean.

Floristic category	Aswan		Qalubya	
	P	A	P	A
Comopolitan	0	5	1	4
Planaeotropical	3	7	4	5
Pantropical	7	12	12	13
Mediterranean	0	2	0	2
ME+IR-TR	0	0	2	1
ME+IR-TR+S-Z	0	0	1	0
ME+SA-SI+S-Z+IR-TR	1	0	0	0
SA-SI	0	0	0	1
SA-SI+S-Z	4	1	2	1
S-Z	3	1	0	1
Total	18	28	22	28
%	39.1	60.9	44	56

The percentage of tropical taxa is higher in Abu Riesh (19.6%) than in El Khanka (10.0%). A high percentage (78.0 and 74.0%) of the weed flora in El Khanka and Abu Reish are represented by the widely spread Cosmopolitan, Palaeotropical and Pantropical taxa. Wickens (1977) found more or less same results.

Seeds collected from studied areas for both *R. communis* and *H. annuus* species showed change in protein band intensity and alternations in their relative mobilities (Table 4 and Fig. 1a&1b).

The electrophoretic patterns for seed proteins of *H. annuus* and *R. communis* species show twenty protein bands for *H. annuus* sample collected from El Khanka compared with 16 bands for that of Abu Riesh area. Of these protein bands 15 are recorded for both samples of *H. annuus* species. On the other hand, 15 protein bands were recorded for each sample of *R. communis* and 13 bands were recorded for both *R. communis* samples. Both plant samples show high sensitivity to sewage water pollution as indicated by the appearance of

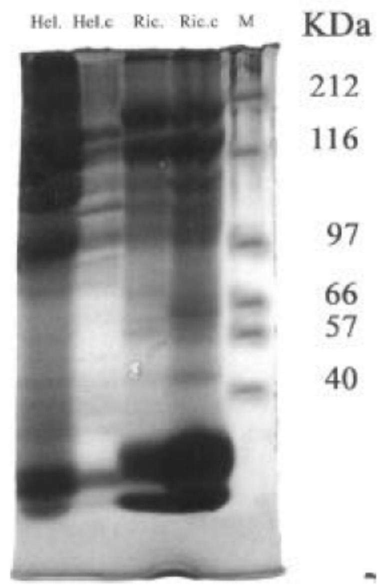

Fig. 1a: Electrophoretic patterns of seed protein using SDS-PAGE for *Helianthus annuus* (Gel. & Hel. c) and *Ricinus communis* (Ric. & Ric. c) in El Khanka and Abu Riesh respectively.

Table 3: Physical and chemical analysis of the soil samples collected from two different provinces (Aswan, Qalubya). Mean and δ-1= standard deviation

Location	Sand (%)	Silt (%)	Clay (%)	Organic matter (%)	pH	EC μmoh/cm	Cl mg/100gm	HCO_3 mg/100gm	Ca mg/100mg	Mg mg/100g (m)
Aswan										
Mean	51.16	15.66	24.99	7.85	7.64	281.5±55.9	7.27±1.75	33.26±2.17	13.63±1.41	5.96±1.03
δ-1	±4.67	±2.92	±0.83	±0.15	±0.15					
El-Hanka										
Mean	87.78	0.62	0.63	7.71	7.71	707±279	15.96±4.13	24.56±1.79	22.90±17.56	6.12±3.15
δ-1	0.74	±0.34	±0.34	±0.29	±0.29					

Table 4: Total protein banding patterns by SDS-PAGE technique analysis using Gel-Pro Analyser V.3 computer program for *Helianthus annuus* (Hel. c) and *Ricinus communis* (Ric. & Ric.) In El Khanka and Abu Riesh respectively.

Lanes Rows	Lane 1 hel. (mol. w.)	Lane 1 hel. Bands%	Lane 1 hel. (amount)	Lane 2 hel. c (mol. w.)	Lane 2 hel. c Bands%	Lane 2 hel. c (amount)	Lane 3 ric. (mol. w.)	Lane 3 ric. Bands%	Lane 3 ric. (amount)	Lane 4 ric. C (mol. w.)	Lane 4 ric. C Bands%	Lane 4 ric. C (amount)	Lane 5 marker (mol.wt)	Lane 5 marker (mol.wt)
r1	345.2	3.21	3.2076				345.2	4.93	4.9283	345.2	3.57	3.5673		
r2	319.62	2.41	2.4075	299.76	2.93	2.9322	299.76	1.86	1.8603					
r3	260.31	2.07	2.0715	263.67	2.37	2.3665								
r4	237.95	3.05	3.0482	228.96	3.64	3.6437								
r5	206.63	2.31	2.3147				198.83	7.26	7.2600	206.63	5.23	5.2284	212	14.84
r6	168.63	4.79	4.7917	168.28	5.18	5.1819	168.28	21	2.1003					
r7	151.87	5.63	5.628	151.87	4.73	4.7309	151.87	7.7	7.7036	151.87	8.24	8.2397		
r8				116	2.36	2.3639							116	14.799
r9	107.73	6.19	6.1931	107.73	5.62	5.6211	107.73	2.87	2.8700	107.73	5.01	5.0102		
r10	104.19	3.89	3.8907	104.19	4.32	4.3221	104.19	4.93	4.9339	104.19	3.06	3.0648		
r11	100.05	6.84	6.8366	100.05	6.54	6.5399	100.05	5	4.9958	100.05	4.70	4.6976		
r12	91.808	2.13	2.1261	5.238	1.76	1.7649							97	16.581
r13	78.559	2.42	2.4209	81.493	1.88	1.8848	85.316	1.87	1.8678	80.012	2.38	2.3755		
r14							73.676	2.89	2.8876	71.023	2.31	2.3054		
r15	64.015	1.9	1.9001				62.47	1.69	1.6887	64.015	2.95	2.9455	66	16.010
r16	58.14	1.9	1.8968				59.128	1.89	1.8927	59.49	1.84	1.8386		
r17	52.863	1.87	1.8625							56.147	1.25	1.2502	57	14.269
r18	45.811	1.85	1.8548	45.811	1.35	1.3507	45.811	1.94	1.9433	45.811	2.85	2.8509		
r19	40.303	2.54	2.544	39.402	1.06	1.0602							40	13.779
r20	32.636	4.33	4.3299	32.636	2.46	2.4588				35.457	1.07	1.0732		
r21	22.391	9.5	9.5017	2.56	7.42	7.4234	22.391	13.7	13.6820	22.391	18.30	18.3330		
r22	19.404	3.47	3.4661	19.404	1.97	1.9663	19.404	6.68	6.6832	19.404	9.89	9.8863		
Sum		72.3	72.295		55.6	55.6120		67.3	67.2980		72.70	72.6670		90.279
In Lane		100	100		100	100		100	100		100	100		100
Σ band		20			16			15			15			

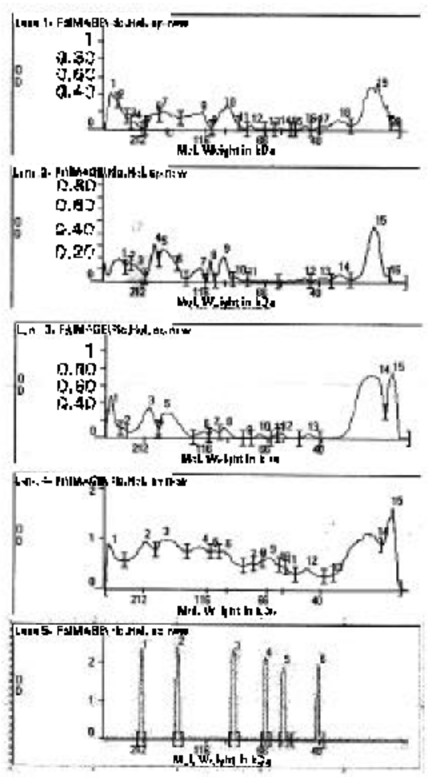

Fig 1b: Graphs of total protein banding patterns by SDS-PAGE technique analysis using Gel-Pro analyzer V.3 computer program for *Helianthus annuus* (Hel. & Hel. c) and *Ricinus communis* (Ric. & Ric.) in El Khanka and Abu Riesh respectively.

novel protein bands about 345.2, 206.63, 64.01,58.41 and 52.86 K.Da. for *H. annuus* and 299.76 and 168.28 for *R. communis*.

The band variation was revealed by alteration in band intensity and appearance and disappearance of some bands. In case of *H. annuus* major variation in the SDS-PAGE is expressed as disappearance of major band (mol. wt. 116.0 K. Da.) in addition to the appearance of novel bands in El-Khanka sample than Abu-Riesh sample. Changes in the electrophoretic pattern were attributed either to alterations in the structural genes or to changes in the expression of regulatory genes involved in regulating the concerned structural gene (Hassan, 1996; Badr *et al*., 1995 & 1998).

The occurrence of additional bands in PAGE profile may be the result of the synthesis of new protein controlled by the mutation (activation) of structural genes. The correlation between the mutation events had been also demonstrated by work of Salamini *et al*., 1979, on *Zea mays*; Prasad and Zha, 1992, on *Phaseolus vulgaris*; Shehab *et al*., 1995, on *Vicia faba*; Soliman, 1998, on *Sesbania sesban* L. and Soliman and Barakat, 1999, on *Leucaena glauca* Benth.

In conclusion, sewage water irrigation can affect soil physical and chemical properties in addition to changing associated weed floras in cultivated areas. *Helianthus annuus* showed higher gene toxicity sensitivity towards sewage water than *Ricinus communis* as indicated by protein banding patterns.

Acknowledgement

Authors express their thanks to Prof. Dr. M. Abdel-Ghany (The Herbarium, Botany Dept. Fac. Sci., Cairo Uni., Egypt.) for identifying *Celosia argentea* specimen and Dr. M. Abdel-Rahman (Geology Dept., Fac. Sci., Helwan Uni., Egypt.) for revising the manuscript.

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