MICROBIOLOGICAL AND PHYSICOCHEMICAL ANALYSIS OF GROUNDWATER AND ITS BIOLOGICAL EFFECT ON POPULATION IN SAINT KATHERINE PROTECTORATE, EGYPT

Ahmed M. Abdel-Azeem[*1], Tamer S. Abdel-Moneim[2], Mohsen E. Ibrahim[3], Mamdouh Y. Saleh[4], Shrief Y. Saleh[5] and Abdel-Moneim O. Abdel-Moneim[6]

[*1] Department of Botany, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt, E-mail: zemo3000@yahoo.com

[2] Department of Agricultural Botany, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt

[3] Department of Geological and Biological Sciences, Faculty of Education, Suez Canal University, Al-Arish, Egypt

[4] Department of Civil Engineering, Faculty of Engineering, Suez Canal University, Port Said, Egypt

[5] Department of Biochemistry, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt

[6] Faculty of Dental and Oral Medicine, Cairo University, El-Kser El-Eni, Cairo, Egypt

ABSTRACT

Water samples were collected from 19 wells (governmental and private) in Saint Katherine protectorate, from June until September 2008. Water samples has been subjected to various physical, chemical and microbiological analyses including water temperature, pH, total dissolved salts (TDS), electric conductivity (EC), NaCl %, nitrite, nitrate, ammonia, total phosphate, heavy metals (Zn, Pb, Cd, Mn and Cu) and Fluoride. Zoosporic and aquatic-derived fungi were isolated from water samples by using baiting technique on different seeds and dilution technique on Czapeks yeast extract agar medium respectively. Total viable and coliform bacteria were isolated by using Nutrient Agar and Endo-agar media respectively. Blood samples were collected from population of this area for determination of serum fluoride, lead, copper, calcium, zinc and phosphorus. Also renal function tests (creatinine, urea, BUN and uric acid) as well as, liver function tests (ALT, AST, ALP, and GGT) were determined in examined blood. An extensive field survey was carried out on 70 children to show the effect of water over fluorosis on their teeth in four settlements.

Fluorides and heavy metals values (Pb, Cu, Mn) of the majority of studied wells water exceed the accepted limits for drinking water standards recommended by Egyptian Ministry of Health (EMH) Decree no. 458/2007. Nitrates and nitrites also exceeded the accepted limits recommended by the EMH and the World Health Organization (WHO) for drinking water standards. Fifteen species of aquatic-derived fungi were identified. The dominant taxa were *Aspergillus niger, A. flavus, A. terrus*, *Mucor racemosus* and *Penicillium chrysogenum*. Eight taxa of aquatic fungi were reported during this study the most dominant were *Achlya prolifera, Saprolegnia diclina* and *Pythium intermedium*. Counts of total coliform reached up to 154700 CFU/100ml in wells. Serum analyses revealed mild increase in serum fluoride and lead above normal range with mild decrease in serum calcium lower than normal range. While kidney and liver functions exhibited as normal. Among the 70 examined children, 100% of infection with different degrees of severity was observed based on the child age as well as the water source.

To minimize the impact of groundwater pollution on human health in Saint Katherine, urgent studies should be carried out on its quality as soon as possible along with an extensive survey of radioactive isotopes which may be decay into stable lead isotopes. On the other hand the hazardous effects of high percentage of fluoride in the groundwater must be controlled or completely eliminated by using the modified model of water purification unit established by Community and Environmental Services Center on El-Zayitona well to provide Saint Katherine inhabitants especially kids with healthy clean glass of water. Finally activation of law 4/1994, for the protection of environment in Egypt, is urgently needed.

Keywords: Saint Katherine, over fluorosis, aquatic fungi, heavy metals, radioactive materials.

INTRODUCTION

Saint Katherine Protectorate covers about 4350 km^2 of Southern Sinai and was declared as protected area by Prime Ministerial Decrees 613/1988 & 940/1996 due to its immense biological and cultural interest. Within this Protectorate, UNESCO at its 26th session in Budapest in June 2002 declared as a World Heritage Site area that coincides with the Saint Katherine ring dyke.

Water is an essential natural resource for sustaining life and environment especially in Egypt as one of arid areas. Groundwater contributes only 0.6% of the total water resources on earth; it is the major and the available source of drinking water in rural as well as urban areas; especially in Saint Katherine, South Sinai area. Bedouins of Saint Katherine consumed 200 m^3/day from the groundwater distributed in approximately 40 wells as the main water source. This vital resource is vulnerable to contamination, and is being increasingly threatened by an array of pollutants from landfills, soil treatment systems, septic tanks and subsurface disposal wells.

Fluoride has been known to be found most frequently in groundwater, its concentration depending on the nature of rocks and natural fluoride carrying minerals at certain depths. Egypt is out of more than 20 developed and developing nations that are endemic for fluorosis. Egyptian Ministry of Health (Decree no. 458 in 2007), determine the fluoride limit in drinking water to be 0.8 mg/L of water. Fluoride content of water wells in Saint Katherine has never been the target of any study before.

Enamel fluorosis is hypomineralisation of enamel characterized by greater surface and subsurface porosity than in normal enamel as a result of excess fluoride intake during the period of enamel formation (Burt and Eklund [1]).The change in the enamel is characterized by altered appearance of the tooth ranging from fine white lines to pitting or staining of enamel. Dean [2] observed a correlation between fluoride in the drinking water and mottled enamel and from this he devised Dean's Index of Fluorosis.

Due to lack of water, the new sewer system of domestic sewage disposal in Saint Katherine city is non-functional and the active system consists of septic tanks and cesspits constructed directly over the fractured-granite and alluvial layers. This is the main reason of migration of different contaminants in such strata (Ghodeif *et al.* [3]; Abdulla *et al.* [4]).

Extensive surveys carried by the scientific team of Community and Environmental Services Center in Saint Katherine (www.saintkatherinecenter.org) during 2007-2008 have showed that the use of groundwater systems in Saint Katherine for household purposes is the main source of health problems especially diarrhea, skin infections, kidney diseases and over fluorosis.

Due to the aforementioned information, the objectives of this study were to survey the quality of groundwater through subjected governmental and private wells to various analyses to determine the microbiological and physicochemical characteristics of groundwater as well as studying the impact of contamination on human health in Saint Katherine Protectorate.

MATERIALS AND METHODS

Study Area

The Saint Katherine region is situated in the southern Sinai and is part of the upper Sinai massif. It is located between 33^o 55' to 34^o 30' East and 28^o 30' to 28^o 35' North. Elevation ranges from 1500 to 2624 meters above sea level which includes the main mountains in the area. Generally, the area is formed of igneous and metamorphic rocks; chiefly granites are intensely dissected and rugged and characterized by outcrops to form several mountain peaks (e.g. Gebel Katherine 2642 m and Gebel Musa 2285 m) (Said [5]). The Saint Katherine area has an extremely arid climate with long, hot and rainless summers and cool winters. The mean annual precipitation in Saint Katherine area over 25 years is 45 mm per year; the high mountains receive more precipitation (100 mm/year) as rain and snow. Temperatures vary from a lowest mean temperature in January of 1.4 °C to a highest mean temperature of 30.8 and 31.8°C in August.

Sampling

Ninety five water samples (5 per each site) were collected from nineteen wells (Table 1, Fig. 1) during the period from June (2008) to September (2008), pumps of the wells were turned on for 30 min before sampling. Samples were collected in sterile well stopper sterilized 1000-ml polyethylene bottles and transferred in an ice box to the laboratory. Microbiological and chemical analyses were carried out within 24 hr of sampling at the laboratories of Community and Environmental Services Center in Saint Katherine. The center (www.saintkatherinecenter.org) is a scientific, research and environmental center established and funded by European Union in 2007 (EuropeAid/123879/C/ACT/EG/127). Blood samples were collected from 20 human population of surveyed area (Group I) to compare with 20 normal healthy persons as control (Group II). Seventy kids from four settlements namely: Shiekh Awad, El-Melqah, El-Tarfa and Wadi Feran respectively were monitored for the effect of over fluorosis on their teeth.

Table 1: GPS readings of the selected nineteen sites in the study area

No.	Name	N	E	Elevation (m)
1	Moustafa Eid Ayed	33 56.816	28 41.714	1181
2	Ayed Eid Ayed	33 56.852	28 41.736	1163
3	Salama Ouda	33 56.796	28 41.676	1163
4	El-Watya*	33 59.316	28 40.406	1298
5	El-Watya*	33 59.360	28 40.371	1291
6	El-Halowgy	33 59.420	28 40.066	1306
7	El-Zayitona*	33 59.503	28 35.681	1443
8	Seel Lodger	33 58.420	28 34.61	1489
9	Tota Well	33 57.424	28 33.489	1579
10	El-Kharaza Well	33 57.358	28 33.469	1567
11	El-Abdaya 1	33 57.349	28 33.518	1549
12	El-Abdaya 2	33 57.361	28 33.535	1554
13	Farag Soliman	33 57.372	28 33.372	1544
14	Ahmed Abu-Rashed	33 57.403	28 33.606	1521
15	Saidy Farag	33 57.420	28 33.658	1527
16	El-Kahla	33 57.532	28 33.828	1523
17	Wadi Sedod 1	33 57.523	28 33.849	1526
18	Wadi Sedod 2	34 00.644	28 33.194	1601
19	El-Hamra	33 59.439	28 33.181	1595

(*) referred to governmental wells.

Figure 1: Satellite image of the different sites in Saint Katherine.

Physico-Chemical Analysis of the Groundwater

Samples has been subjected to various analyses including water temperature at the time of sampling using a mercury thermometer; pH value (using portable pH meter, model HI 8314 Hanna Ins. Romania), total dissolved salts (TDS), electric conductivity (EC), NaCl %, (using microprocessor EC/TDS meter HI 98360 Hanna Inst., Romania). Heavy metals (Zn, Pb, Cd, Mn and Cu) were analyzed by the total adsorbed metals method according to USEPA [6] using atomic spectrophotometer (model PYE UNICAM SP9, England). Estimation of phosphate, nitrate, nitrite, and ammonia were analyzed according to standard methods for examination of water and wastewater (APHA [7]).

Microbiological Analyses

For the recovery of zoosporic fungi, the baiting technique adopted by El-Hissy and Khallil [8] was used. The seeded plates (5 plates for each sample) were incubated at 22°C for two weeks during which the growing colonies were examined and identified. For the determination of fungal population, the zoosporic fungal species appearing on one plate was counted as one colony. Relevant references for taxonomic identification of isolated zoosporic fungi genera and species were used such as Coker [9], for Saprolegniaceae and other water moulds, Johnson [10], for *Achlya* species, Sparrow

[11], for aquatic phycomycetes in general, Waterhouse [12, 13], for *Pythium* species, Seymour [14], for *Saprolegnia* species. Aquatic-derived fungi were isolated on Czapek's agar supplemented with 0.5 % yeast extract (CYA), amended with Rose bengal (1/15000) and chloramphenicol (50 ppm) for primary isolation, isolation plates (five plates/site) were incubated for one week at 28 ^{o}C. Taxonomic identification using morphology characteristics of fungal isolates down to the species level on standard media was mainly based on the following identification keys: Raper & Thom [15], Pitt [16] for *Penicillium*; Raper & Fennell [17] for *Aspergillus*; Booth [18] for *Fusarium*; Arx [19], Domsch *et al.* [20], Watanabe [21] for miscellaneous fungi; Arx *et al.* [22] for *Chaetomium*. The systematic arrangement follows the latest system of classification appearing in the 9th edition of Anisworth & Bisby's Dictionary of the fungi (Kirk *et al.* [23]) and Index Fungorum web site (http://www.indexfungorum.org/Names/Names.asp).

Total viable bacteria were enumerated in water samples after having been serially diluted in saline sterilized solution and plated onto nutrient agar plates. Total coliform bacteria were determined using the membrane-filter technique (Greenberg *et al.* [24]). One hundred milliliters of each sample were aseptically filtered through sterile 0.45µm-pore size membrane filters (Whatman), and the filters transferred onto Endo-agar medium (MacFaddin [25]). Total viable bacteria plates were incubated at 28°C; and total coliform plates at 37°C. All bacterial groups were counted after 24 hr of incubation.

Blood Analyses

Blood samples were left to clot at room temperature then centrifuged at 3000 r.p.m for 15 minutes for separation of serum. Sera were kept at -20 C till determination of biochemical parameters. Serum urea, Blood Urea Nitrogen (BUN) ,creatinine, uric acid, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) and Gamma-glutamyl transpeptidase (GGT) were determined colorimetrically using commercial kits (Bio-Merieux Co. Maray-Le toile, Chrobonnieres-Les Bains, France) according to the methods of Fawcett and Scott [26]; Seeling and Wust [27]; Barham and Trinder [28]; Wallnofer *et al.*, [29]; Tietz, [30] and Persijin and Van der Slik [31] respectively. Serum calcium was determined colorimetrically by Perkin-Elimer atomic absorption spectrophotometer, using commercial kits (Human, Gesell Schaft for Biochemical and Diagnostic, Wiesbaden, Germany) according to the method of Barnett *et al.* [32]. Inorganic phosphorus was determined colorimetrically using kits supplied by Biomerieux, France and according to the method of Goldenberg and Fernands [33]. Trace elements (zinc, copper, lead and fluoride) were determined by using atomic absorption spectrophotometery on analyst by Berkin Elmart Company.

Fluoride and dental fluorosis measurements

Fluoride concentration in the samples was measured by a fluoride selective electrode and a reference electrode. The type used was a Radiometer F1052 fluoride electrode and Metrohm Ag/AgCl reference electrode with sleeve type diaphragm connected to a Metrohm 691 potentiometer. The fluoride is measured after addition of TISAB according to APHA [7]. The obtained mV-reading is compared with a standard curve made from measurement of solutions of known fluoride concentrations. The used reagents and the procedure are described more thoroughly in the "Instruction Manual for Determination of Fluoride in Water Samples", Bregnhøj [34]. Dental fluorosis observed during this survey was grouped according to Dean's Index fluorosis grades as recommended by Browne *et al.* [35].

Data Analyses

All data were statistically analyzed using student "t" test according to Snedecor and Cochran [36] using a Costate statistical package (Anonymous [37]).

RESULTS

Physico-chemical parameters

Data of Table (2) showed some physicochemical parameters of the examined samples. Values of pH, temperature and TDS of water samples were within the accepted limits and variations among them is not significant ($P>0.05$).

However, water showed high significant differences among wells ($P<0.001$) in their salt content, as indicated by values of electric conductivity, NaCl % and total dissolved salts. Wells 4, 6 and 7 were far from the acceptability standards in their salt contents.

Nitrogen and phosphorous values were significantly different among the 19 wells ($P < 0.05$). Levels of nitrite nitrogen in particular were very far from the recommended levels in 79% of the wells, being only acceptable in wells (1, 14, 15 and 16). Nitrates level is acceptable in fifteen wells out of nineteen. Ammonia and phosphorus levels were acceptable in all wells varying between 0.0–0.258 mg/l for ammonia and 0.102–1.302 mg/l for phosphorous.

Levels of the examined heavy metals (zinc, copper and manganese) were not significantly different among the wells ($P>0.05$) and were in agreement with national and international standards (Table 3). On the other hand cadmium and lead concentrations showed high significant difference among wells ($P<0.001$). Out of 19 wells, fifteen were contaminated by lead and were far from the acceptable limit of standards and five by cadmium.

Eighteen wells were contaminated with fluoride with levels exceed the permissible limits of EMH varying between 0.9 -2.004 mg/l. These concentrations showed high significant difference among all wells ($P<0.001$).

Microbiological analyses

Fifteen isolated species of aquatic derived fungi belonged to eleven genera were recovered from 19 water samples. The prevailing genera were *Aspergillus* (4 species, 26.6% of the total isolate number) and *Mucor* (2 species, 13.3 %). All the remaining genera were represented by one to three species each (Table 4).

Eight species which belong to five zoosporic fungal genera were recovered from 19 water samples during this investigation. In addition 77 colonies were encountered and belonged to five genera namely, *Achlya* (38 colonies), *Dictyuchus* (2 colonies), *Isoachlya* (3 colonies), *Pythium* (9 colonies) and *Saprolegnia* (25) (Table 4). The water samples (19) contributed 7 identified species in addition to 1 unidentified species. Genera namely, *Achlya* and *Pythium* were represented only by 2 species. The remaining genera were represented only by one species each. *Achlya prolifera* was the commonest identified species and were represented in 84.2 % of total samples. The remaining species were of low or rare occurrence (Table 4).

Taxonomically, the species isolated were assigned to fifteen genera, twenty two species, eight families, six orders, four classes and three phyla and two kingdoms (Table 5). Family Trichocomaceae had the highest contribution to the mycobiota (7 species out of 22) followed by Saprolegniaceae (6), Mucoraceae (3), while the remaining families were represented only by one species each.

Bacteriological analysis indicated that total viable bacteria (TVB) counts ranging between $1.16x10^{3}$ to $28x10^{4}$ cfu/100ml, which exceeds the acceptable limits (100 CFU/100ml) in all the investigated wells. In addition, total coliform (TC) bacteria were detected in all wells except No. 18 with values up to 154700 CFU/100ml (Figure 2 a, b).

Blood Biochemical Parameters

All results revealed mild increase in serum fluoride and lead above normal range with mild decrease in serum calcium lower than normal range. While kidney and liver functions exhibited as normal. The mild increase in serum fluoride in spite of its exceed the accepted limits for drinking water recommended by Egyptian Ministry of Health, may attributed to the normal renal function as shown in (Table 6) which help the individuals to excrete the fluoride in their urine. The obtained results in present survey showed mild increase in serum lead with mild decrease in serum calcium. This result may be due to direct effect of exceeded lead in drinking water when taken to individuals on calcium metabolism.

Enamel fluorosis

Due to excessive fluoride intake of the children their teeth enamel shows the symptoms of dental fluorosis (Table 7). Low severity of enamel fluorosis was observed as the teeth lose its luster in the children with age ranges from 3 to 6 years old, in Shiekh Awad and El Melqah settlements while mild fluorosis severity observed in all settlements especially in the children with age group >6 - <10. The symptoms appear as opaque areas on the tooth surface.

The severe form of enamel fluorosis is manifested as yellowish brown to black stains and severe pitting of the teeth, also porosity increases and lesions extend toward the inner enamel. This type observed in children of > 10-14 years old in Shiekh Awad; as well as children group of >6 - <10 years old in El Tarfa, El Melqah and Wadi Feran settlements (Table 7). Among the severe cases observed in El-Tarfa children, Saint Katherine (Figure 3 a, b), the appearance of brown discolored teeth and crippling of enamel as symptoms of extremely severe enamel fluorosis was clearly observed.

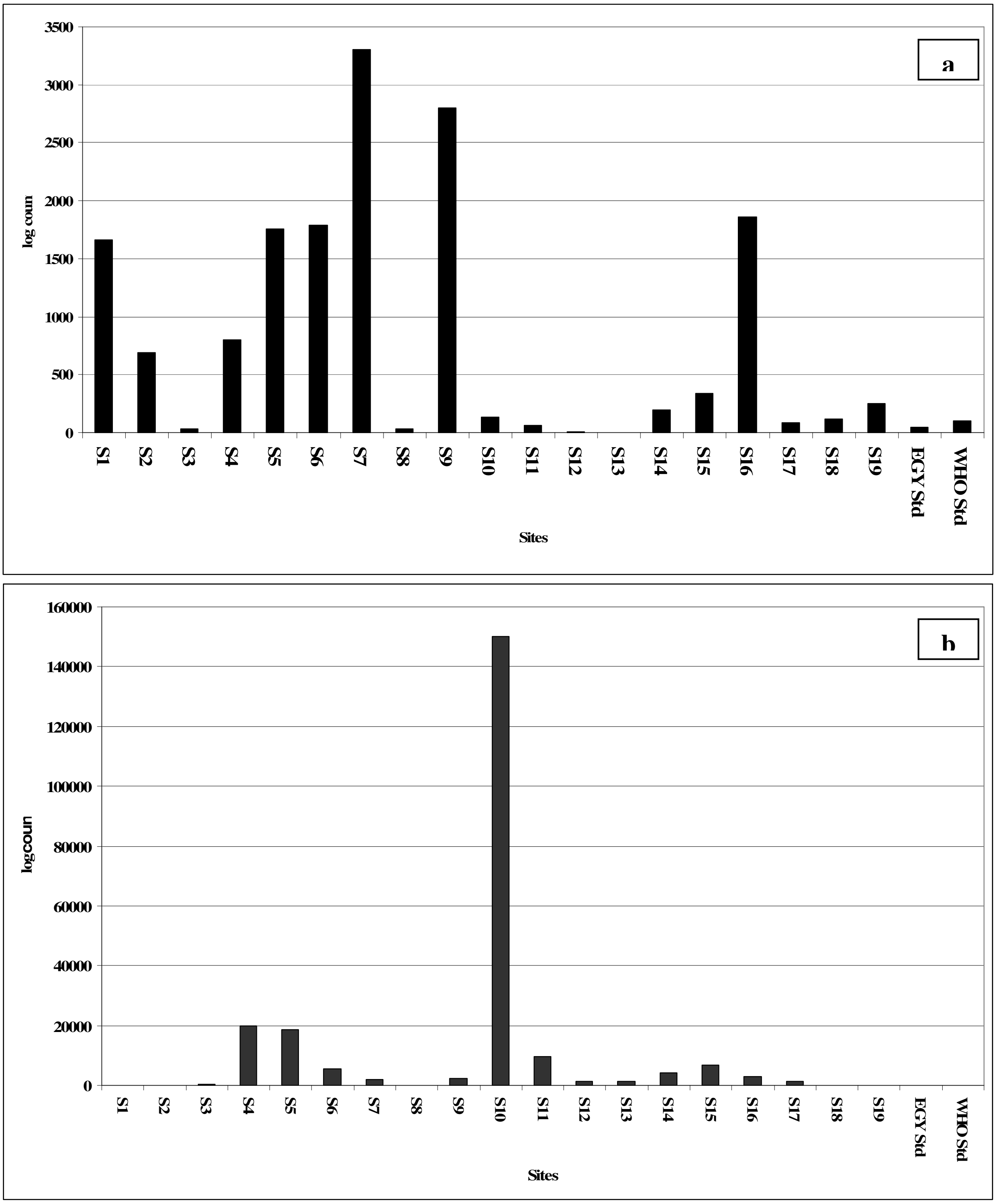

Figure 2: A: Counts of total viable bacteria (CFU/1ml) in water samples collected from the studied 19 sites in Saint Katherine protectorate. B: Counts of total coliform (CFU/100 ml) compared to the Egyptian standards of acceptability for potable water. The guideline values for total coliform are all 0 CFU/100ml, and for TVB are 50 CFU/ml and 100 CFU/ml respectively according to Egyptian and WHO standards.

Normally, the degree of dental fluorosis depends on the amount of fluoride exposure up to the age of 8–10, as fluoride stains only the developing teeth while they are being formed in the jawbones and are still under the gums. The effect of dental fluorosis may not be apparent if the teeth are already fully grown prior to the fluoride over exposure. Therefore, the fact that an adult shows no signs of dental fluorosis does not necessarily mean that his or her fluoride intake is within the safety limit.

Figure 3: A: Child in El-Tarfa, Saint Katherine with brown discolored teeth and crippling enamel as symptoms of dental fluorosis. B: Examination of a Kid during the extensive survey carried by the dentist.

Table 2: Physicochemical parameters of water samples collected from 19 wells compared to the Egyptian and WHO standards for potable water. EGY Std = Egyptian acceptable limit; WHO Std = World Health Organization acceptable limit; NSV = no acceptable limits set and (*) referred to parameters exceed the Std.

Parameter		pH	Temp	EC (uS/Cm)	TDS (mg/L)	NaCl%	Nitrate (mg/l)	Nitrite (mg/l)	Ammonia (mg/l)	Phosphate (mg/l)
Site No.	1	7.7 ±0.05	25	633±0.03	319±1.3	1.35±0.09	3.8±0.08	0.132±0.006	0	0.102±0.0003
	2	7.81±0.047	26	568±0.05	285±0.7	1.004±0.77	3.5±0.07	0.902*±0.005	0	0.11±0.006
	3	7.69±0.021	25.9	581±0.34	291±0.56	1.062±0.05	3.778±0.5	0.24*±0.0008	0	0.142±0.007
	4	7.86±0.019	25	860±0.5	433±2.0	1.54±0.01	128*±2.5	3.1*±0.0009	0.09±0.0003	1.302±0.009
	5	8.02±0.123	26	696±1.5	348±3.44	1.364±0.003	155.4*±0.9	2.796*±000.7	0.226±0.0006	1.092±0.0008
	6	7.66±0.045	25.5	1114±2.7	558±2.34	2.126±0.003	143*±2.6	1.194*±0.0009	0.176±0.0005	0.98±0.0008
	7	7.78±0.024	26	806±3.2	403±4.0	1.56±0.02	177*±3.4	0.796*±0.003	0.258±0.0005	1.3±0.007
	8	8.18±0.044	25.3	401±0.7	201±0.09	0.732±0.04	12±1.1	0.506*±0.005	0	0.48±0.0078
	9	7.83±0.153	26.6	350±0.9	175±0.6	0.658±0.001	17±0.05	0.374*±0.0009	0	0.26±0.00056
	10	8.91±0.008	26.5	513±2.0	257±0.35	1.06±0.001	23±0.06	0.45*±0.01	0	0.206±0.00057
	11	7.89±0.022	26.1	318±1.1	159±0.24	0.634±0.001	17±1.4	0.336*±0.07	0	0.132±0.00007
	12	7.75±0.006	25.8	344±3.4	172±0.22	0.71±0.006	15±0.009	0.22*±0.05	0.072±0.0004	0.116±0.0007
	13	7.7±0.028	26.5	382±2.4	191±0.44	0.804±0.004	13±0.09	0.208*±0.007	0.066±0.0034	0.434±0.0006
	14	7.5±0.094	26.5	370±1.3	185±2.7	0.808±0.0009	10±0.08	0.18±0.0071	0.058±0.00046	0.138±0.0005
	15	7.47±0.033	27.1	404±2.2	201±0.55	0.804±0.008	12±0.04	0.16±0.0003	0	0.334±0.00067
	16	7.63±0.010	27.2	315±0.04	157±4.35	0.648±0007	11±0.02	0.17±0.006	0.082±0.007	0.816±0.0045
	17	7.95±0.005	23.9	668±o.9	333±2.9	1.328±0.0004	3.988±	0.702*±0.007	0	0.286±0.0006
	18	7.74±0.005	26.7	447±0.77	323±0.98	0.91±0.005	5.09±	0.324*±0.006	0	0.302±0.00098
	19	7.45±0.028	24.2	611±0.34	305±0.58	1.08±0.002	7.5±	0.506*±0.002	0	0.154±0.0067
EGY Std		6.5 – 8.5	22	NSV	1000	NSV	45	0.2	0.5	NSV
WHO Std		6.5 – 8.5	NSV	NSV	1000	NSV	10	3	Nil	NSV

Table 3: Heavy metals and fluoride contents of water samples collected from 19 wells compared to the Egyptian and WHO standards for potable water. EGY Std = Egyptian acceptable limit; WHO Std = World Health Organization acceptable limit and (*) referred to parameters exceed the Std

Parameter		Heavy Metals (mg/ L)					F (mg/ l)
		Cd	**Pb**	**Zn**	**Cu**	**Mn**	
Site No.	1	0.0016±0.00010	0.03136*±0.0003	0.00712±0.0002	0.0053±0.0006	0.00582±9.7 x10^{-5}	1.1*±0.063
	2	0.0014±7.7 x10^{-5}	0.029*±0.0005	0.008±0.0003	0.00456±0.0001	0.00662±0.0002	1.1*±0.066
	3	0.0017±4.47 x10^{-5}	0.03236*±9.8 x10^{-5}	0.00664±0.00016	0.00578±0.00012	0.00628±0.0002	1.226*±0.020
	4	0.0048*±3.16 x10^{-5}	0.01026*±6.7 x10^{-5}	0.0024±0.0007	0.00494±0.0003	0.00422±0.0001	1.888*±0.077
	5	0.0012±7.07 x10^{-5}	0.01234*±0.0001	0.00358±0.0009	0.00326±0.0001	0.0047±7.07 x10^{-5}	1.554*±0.027
	6	0.0044*±3.16 x10^{-5}	0.0192*±0.0006	0.00498±0.00096	0.03176±0.0012	0.06688±0.0004	1.69*±0.041
	7	0.0015±7.07 x10^{-5}	0.0172*±0.0009	0.00574±0.0003	0.0048±0.0002	0.454*±0.014	2.004*±0.157
	8	0.00248±0.000115	0.00174±0.0001	0.00686±0.0002	0.0077±0.0002	0.00582±0.0002	1.432*±0.039
	9	0.00132±5.8 x10^{-5}	0.00488±0.0004	0.00548±0.0002	0.00422±0.0003	0.0069±0.0002	1.336*±0.035
	10	0.0022±0.0005	0.0176*±0.0011	0.00652±0.0002	0.01374±0.0005	0.00574±0.0002	1.23*±0.056
	11	0.00072±5.8 x10^{-5}	0.0045±7.7 x10-5	0.00792±0.0004	0.00168±0.0001	0.0008±7.07x10^{-5}	0.79±0.042
	12	0.004*±0.0003	0.00432±0.0001	0.00868± 8 x10^{-5}	0.01106±0.0003	0.00332±0.0001	1.102*±0.085
	13	0.002±7.7 x10^{-5}	0.03152*±0.0001	0.0076±0.0004	0.00434±0.0002	0.01192±0.0002	1.304*±0.039
	14	0.00092±3.7 x10^{-5}	0.0378*±0.0004	0.0076±0.0001	0.0037±0.0002	0.01736±0.0006	0.9*±0.035
	15	0.0024±0.0001	0.0355*±0.0009	0.00684±0.0002	0.00226±6.7 x10^{-5}	0.0059±0.0002	1.178*±0.086
	16	0.0013±8.3 x10^{-5}	0.03476*±0.0006	0.00746±0.0001	0.001±0	0.00972±0.0005	1.326*±0.112
	17	0.00322*±0.0001	0.06152*±0.0006	0.0092±0.0002	0.00244±0.0001	0.00516±0.0002	1.448*±0.044
	18	0.0038*±0.0008	0.0489*±0.0004	0.00754±0.0001	0.000894±2.9x10^{-5}	0.0034±0.0002	1.47*±0.070
	19	0.003±0.0003	0.04582*±0.0002	0.0085±0.0001	0.00264±5.1 x10^{-5}	0.0183±0.0002	0.932*±0.031
EGY Std		0.003	0.01	3	2	0.4	0.8
WHO Std		0.003	0.01	NSV	2	0.4	1.5

Table 4: Total counts (TC), number of cases of isolation (NCI) and occurrence remarks (OR) of aquatic and aquatic-derived fungi isolated from 19 water wells in Saint Katherine

Species list	Site No.																					
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**TC**	**NCI**	**OR**
Aquatic-derived fungi																						
Aspergillus flavus Link	10	12	5	8	11	9	17	2	0	6	9	3	12	8	0	12	11	10	8	**153**	**17**	**H**
A. niger var. niger Tiegh.	15	22	7	9	18	13	13	9	13	7	3	5	8	11	15	21	3	5	12	**209**	**19**	**H**
A. terreus Thom	0	0	0	3	0	0	0	5	0	0	0	2	0	0	0	7	0	0	0	**17**	**4**	**L**
A. versicolor (Vuill.) Tirab.	0	3	4	3	3	0	0	0	0	0	0	0	5	0	0	0	0	0	4	**22**	**6**	**M**
Chaetomium globosum Kunze	0	0	0	2	0	0	5	0	0	0	0	0	4	6	0	0	0	0	0	**17**	**4**	**L**
Emericella nidulans (Eidam) Vuill.	2	0	0	0	0	2	1	4	0	0	0	0	0	3	1	0	0	0	0	**13**	**6**	**M**
Fusarium solani (Mart.) Sacc.	2	2	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	**7**	**5**	**M**
Mucor circinelloid Tiegh.	0	0	2	0	0	2	0	0	0	0	0	3	0	0	0	0	0	1	0	**8**	**4**	**L**
M. racemosus Fresen.	3	0	0	3	0	0	0	3	3	3	0	0	0	1	1	1	1	0	0	**19**	**9**	**M**
Mycocladus corymbiferus (Cohn) J.H. Mirza	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	**3**	**3**	**L**
Pencillium chrysogenum var. ***chrysogenum*** Thom	0	0	0	0	0	0	1	9	1	1	0	11	3	5	0	0	7	0	0	**38**	**8**	**M**
Paecilomyces varioti Bainier	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	**8**	**1**	**R**
Rhizopus stolonifer var. ***stolonifer*** (Ehrenb.) Vuill.	0	4	0	0	0	0	0	5	0	0	0	3	0	0	0	1	0	0	1	**14**	**5**	**M**
Trichoderma pseudokoningii Rifai	0	0	0	0	0	5	3	0	1	0	0	0	2	0	0	0	0	0	0	**11**	**4**	**L**
Yeast	1	0	0	1	2	0	0	0	1	0	1	1	0	5	0	7	0	4	3	**26**	**10**	**H**
Aquatic fungi																						
Achlya prolifera C. G. Nees	2	1	3	4	4	2	1	1	0	3	3	2	1	0	1	0	3	2	1	34	**16**	**H**
Achlya racemosa Hildebrand	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	1	4	**3**	**L**
Dictyuchus sterile Coker	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	2	**2**	**R**
Isoachlya monilifera (de Bary) Kauffmann	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	3	**2**	**R**
Pythium intermedium de Bary	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	3	**3**	**L**
P. irregulare Bouisman	0	0	2	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	6	**3**	**L**
Saprolegina diclina Humhrey	0	0	0	0	0	2	0	0	3	0	0	0	0	0	0	3	0	4	0	12	**4**	**L**
Saprolegnia sp.	0	0	0	2	0	0	1	0	0	1	0	0	0	1	3	0	2	3	0	13	**5**	**M**

Occurrence remarks: High (H): More than 50% of NCI; Moderate (M): More than 25% -50% of NCI, Low (L): More than 12.5-25% of NCI, Rare (R): Less than 12.5% of NCI.

Table 5: Systematic diversity of the isolated taxa of fungi from 19 water wells

Kingdom	Phylum	Class	Order	Number of Taxa	
				Genera	Species & varieties
Fungi	Ascomycota	Eurotiomycetes	1	4	7
		Sordariomycetes	2	3	3
		Total	3	7	10
	Zygomycota	Zygomycetes	1	3	4
		Total	1	3	4
Chromista	Oomycota	Oomycetes	2	5	7
		Total	2	5	7
Total			6	15	22

Table 6: Some blood biochemical parameters of population from studied area as compared with normal control population

Parameters	Group I n=20	Group II n=20
Liver functions		
ALT (U/L)	22.4±10.5	20.0±9.5
AST (U/L)	20.4±6.6	16.5±4.1
GGT (U/L)	22±9.3	30.3±5.3
ALP (U/L)	45±4.6	55.4±6.5
Kidney function tests		
Urea (mg/dl)	25.8±5.4	30.0±3.1
BUN	12.06±2.55	13.4±1.6
Creatinine (mg/dl)	0.75±0.23	1.0±0.2
Uric acid (mg/dl)	3.04±0.59	5.4±0.56
Serum metal and electrolytes		
Fluoride (ug/dl) ·	1.4 ± 0.21 [a]	0.9±0.08 [b]
Lead (ug/dl) ·	10.83±2.3 [a]	9.0±0.78 [b]
Copper, (ug/dl)	89.8±15	100.1±9.0
Calcium, (mg/dl).	6.88±0.78 [a]	8.9±0.65 [b]
Phosphorus (mg/dl).	3.78±0.56	3.4±0.23
Zinc (ug/dl)	69.2±7.4	80.1±7.6

a,b means in the same row with no common superscript are significantly different at ($P < 0.05$).

Table 7: Over fluorosis in a sample composed of 70 children in four settlements in Saint Katherine Protectorate

Settlement	Child age (Year)	Severity of enamel fluorosis		
		Low	Mild	Severe
Shiekh Awad				
	3-6	2		
	>6 - <10		6	
	> 10-14			8
El- Melqah				
	3-6	2		
	>6 - <10	6	4	2
	> 10-14			2
El- Tarfa				
	3-6		2	
	>6 - <10		4	6
	> 10-14		14	*
Wadi Feran				
	3-6		1	
	>6 - <10		2	4
	> 10-14			5
		10	**33**	**27**

(*) No sample taken

Water purification unit

Saleh [38] designed and installed the water purification unit on El-Zayitona well in Saint Katherine as one of the major objectives of the Community and Environmental Services Center project. The unit is producing 10 m^3/ day of highly purified drinking water. It composed of alum sedimentation basin (with alum concentration, 50-80 mg/L), motor pump, sand filter, ultraviolet unit for disinfection (wave length 254 nm), solar energy unit for desalination, chlorinator (1-1.8 mg/L) and ozonolizer (1200L/hr) and finally stored in PVC tank (Figure 4).

According to the high percentage of fluoride in the groundwater in Saint Katherine Protectorate a modification in the design of purification unit has been adopted. This modification is based on mixing lime solution with groundwater at 80^{o}C for 10 minutes with a concentration up to 100 mg/l as mentioned by Dahi *et al.* [39].

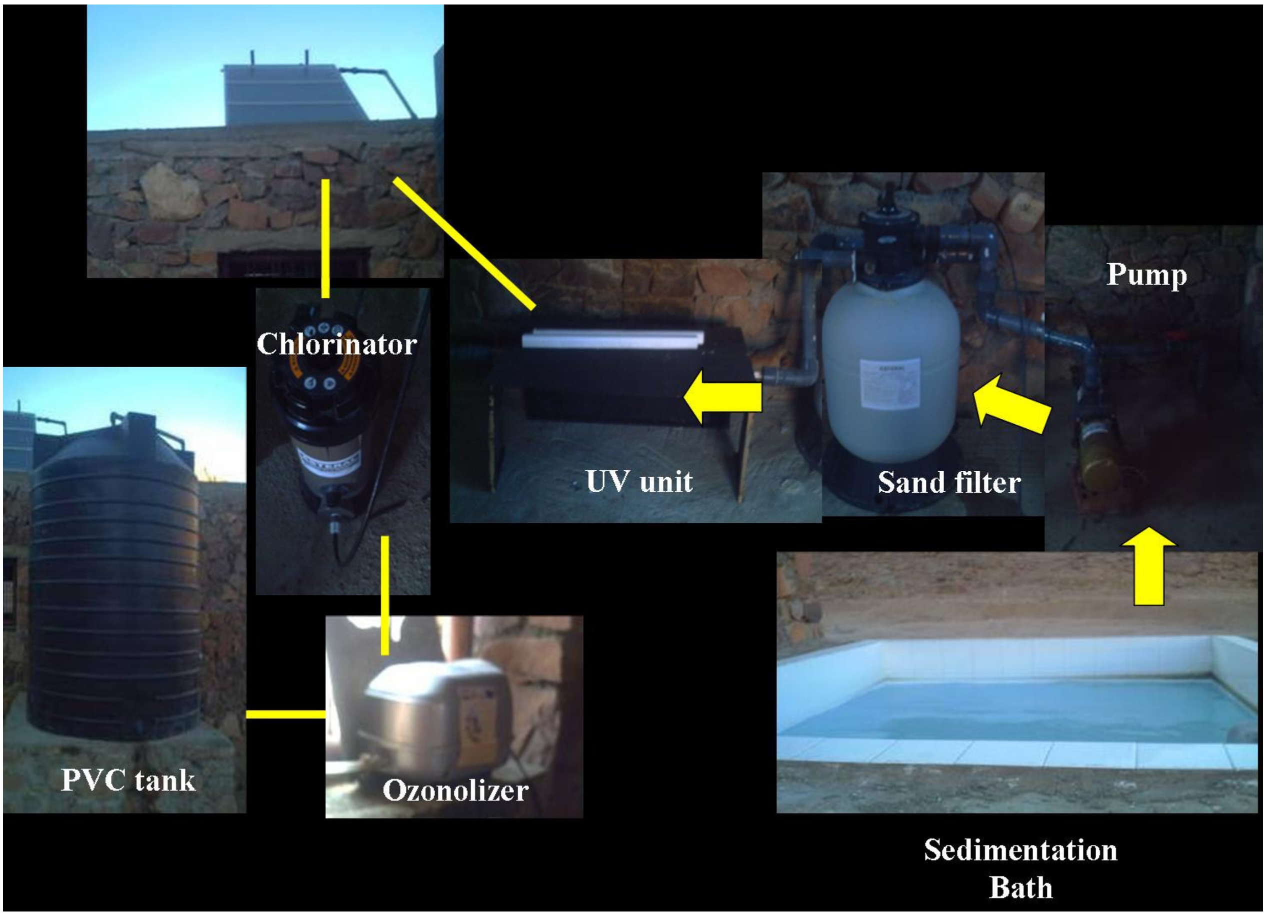

Figure 4: A collage of the purification unit designed and installed by Saleh, M. [38] on El-Zayitona well in Saint Katherine

DISCUSSION

Groundwater pollution, often due to contaminant seepage from the disposal sites, is a big problem in many countries and contamination of groundwater in Saint Katherine is turned one of the major problems facing the local inhabitants and the government. Two major obstacles confront those responsible for water management in Saint Katherine and South Sinai: groundwater contamination of the main aquifer and a shortage of groundwater. The discharge of sewage at the subsurface level is uncontrolled. Sewage is only partly collected and discharged to the natural drainage system.

Water borne diseases are diseases spread through contaminated water either by direct contamination (point source pollution), such as an aquifer recharging with poor quality wastewater effluent or indirect point source contamination comes from wastewater reuse in agriculture or seepage from networks or open systems. Both types of contamination were recorded in Saint Katherine by various investigators like Diab [40] and Abdulla *et al.* [4]. Survey studies carried by Community and Environmental Services Center staff during the period from 2007 to 2008 indicate that enteric/respiratory and eye diseases can be associated with and caused by the use of water contaminated with pathogenic bacteria and viruses resulting from exposure to pollution from domestic wastewater sources. These findings are in agreement with WHO [41].

The total count of viable bacteria and coliform is very high, compared to those observed by Diab [40] and Abdulla *et al.* [4]. This is quite obvious when our results are compared with the relevant work of Diab [40]) and Abdulla *et al.* [4], since the numbers of coliform bacteria have increased in some wells by up to 1000-fold within twenty four years. Keswick [42] reported that fifty percent of waterborne diseases are attributable to contaminated groundwater. The present data indicate that groundwater quality in Saint Katherine is getting more contaminated with time.

As a result of disposal systems such as septic tanks and cesspits the nitrogen and phosphorus concentration may exceeding the acceptable limits of groundwater (Entry & Farmer [43], WHO [44]) and can cause serious diseases in infants if exceed the range of 10 mg/l (Ward *et al.* [45]) Groundwater in some wells of Saint Katherine suffers from very high nitrate levels, reaching up to 177 mg/l and the currently estimated level as same as determined by Abdulla *et al.* [4] and seven-fold increase in nitrate pollution after a further ten years (Shendi & Geriesh [46]) is probably related to the rapid population growth and improper sewage disposal.

Concentrations of heavy metals especially lead; cadmium and manganese are exceeding the Egyptian and WHO standards in the majority of the studies wells. This may be referred to either high concentration of heavy metals in landfill in Saint Katherine or due to their transformations after transportation and can have a large environmental, public health and economic impact (Nriagu and Pacyna [47]; Gadd and White [48]).

On the other hand the high concentration of lead in the groundwater is a warning tocsin because some radioactive isotopes may be decay into stable lead isotopes (Babu *et al*. [49]).

The sewage system in Saint Katherine city consists of septic tanks and cesspits constructed directly over the fractured-granite and alluvial layers (Ghodeif and El-Shafey [50]). So the detection of contaminants in groundwater may be due to direct passage of sewage through wide fracture openings in the granite without filtration.

This is the first study concerning aquatic and aquatic derived fungi in Saint Katherine. Some of the isolated taxa have the ability for producing mycotoxins *viz*. *Aspergillus niger*, *A. flavus*, *Fusarium solani* Susan Lillard [51] and this another hazardous source of groundwater contamination in Saint Katherine.

In the current study, all evaluated wells were contaminated, despite varying in degree of contamination. According to the local Egyptian and international WHO guidelines, water derived from these wells is heavily contaminated with heavy metals, bacteria and fungi.

Serum analyses revealed mild increase in serum fluoride and lead above normal range with mild decrease in serum calcium lower than normal range. Schiffl [52] reported that, patients with chronic renal insufficiency are at an increased risk of chronic fluoride toxicity. Patients with reduced glomerular filtration rates have a decreased ability to excrete fluoride in the urine. These patients may develop skeletal fluorosis even at 1 ppm fluoride in the drinking water. In addition Bansal and Tiwari [53] reported that, individuals with kidney disease have decreased ability to excrete fluoride in urine and are at risk of developing fluorosis even at normal recommended limit of 0.7 to 1.2 mg/l. Moreover, Arnala, [54] mention that, the fluoridation of drinking water up to 1.2 ppm apparently does not pose a potential risk to bone provided the renal function is normal. We should however, recognize that it is difficult to give a strict value for a safe fluoride concentration in drinking water, because individual susceptibility to fluoride varies.

On the other hand Paul and Larry [55] reported that, blood lead levels will frequently be normal in spite of chronic lead toxicity. This occurs because lead is deposited primarily in bones and the brain, so that only minimal levels of lead remain in the blood approximately thirty days after exposure to lead. Julian and O'Hara [56] mentioned that a low tissue calcium level is frequently associated with an elevated lead level. Low calcium to magnesium ratio is also frequently associated with an elevated lead level. An elevated lead level is frequently a causative factor of calcium biounavailability.

Enamel fluorosis is a universal problem in certain high water fluoride areas in Africa and Europe (Forsman [57] and Opinya *et al.* [58]). During this study mild to severe enamel fluorosis was observed in the teeth particularly the primary second molars, as reported in Sheikh Awad, EL Melqah, El Tarfa and Wadi Feran communities. Similar results were obtained by Browne *et al.* [35].as they concluded that the majority of the fluorosis in primary teeth was confined to the second deciduous molars as compared to the first deciduous molars.

In spite of, the critical period at which teeth are most at risk of developing enamel fluorosis: 15–24 months of age for males and 21–30 months of age for females Browne *et al.* [35]. This study is not including this age range; however the presence of this case is expected in the studied communities; related to their dependence on well's water for daily consumption. Enamel fluorosis is severely affecting during the pre-eruptive phase, as the teeth are developed and enamel calcification occurs (Browne *et al.* [35]).

CONCLUSION

Groundwater in this area cannot be considered safe for drinking unless properly treated. To achieve this safety we recommend the following:

- Recognition of the existence of the problem and don't blackout either by the decision makers or the multimedia.
- A monitoring program should be initiated for the St. Katherine area, aiming to study the effect of over fluorosis on the human health.
- Defluoridation using the Nalgonda Technique by establishment of modified purification units by the government on all water wells.
- Activation of environment law 4/1994 against hotels, tourist camps which watering plants by black water.
- Apply a suitable technique for wastewater recycling through construction of wetland system of the Gravel Bed Hydroponic (GBH) type is highly recommended.
- Enhancement of natural aquifer recharge through establishing simple traditional retardation dams for floodwater in Wadi Sedod, El-Arbaein and/or Wadi El-Talaa (upstream parts away from the contaminated areas).
- Further study is recommended to identify risk factors in order to reduce the prevalence of enamel fluorosis and employ methods to manage such risk factors.
- An extensive survey by a team from Authority of Nuclear Materials is highly recommended to measure the level of radiation in the ground water wells in Saint Katherine.

ACKNOWLEDGMENTS

This work has been produced with the financial assistance of the European Union (EuropeAid/123879/C/ACT/EG/127). The contents of this work are the sole responsibility of Community and Environmental Services Center in Saint Katherine and can under no circumstances be regarded as reflecting the position of the European Union. We would like to thank Mr. Bassem Balbol, Mr. Akram Hassan, Mr. Ahmed Atef and Mr. Hussien Farouk (Community and environmental Services center in Saint Katherine) for their valuable helps during this study.

REFERENCES

[1] Burt, B.A. and Eklund, S.A. Dentistry, dental practice and the community, 4th ed. Philadelphia: WB Saunders, p. 147, 1992.

[2] Dean, H.T. Classification of mottled enamel diagnosis. Journal of the American Dental Association; 21:1421–6, 1934.

[3] Ghodeif, K., Dragon, K. and Gorski, J. Ground water quality in the Arabian-Nubian shield, Mount Sinai, Egypt. Geologos 4: 30-46, 1999.

[4] Abdulla, H., Ghodeif, K., El-Shatoury, S. and Dewedar, A. Potential contamination of groundwater in the World Heritage Site of the St. Katherine Protectorate, Egypt. Egyptian Journal of Biology, Vol. 5, pp 1-9, 2003.

[5] Said, R. The Geology of Egypt, Rotterdam, Netherlands, A. A. Balkema Publishers, p. 734, 1990.

[6] U. S. Environmental Protection Agency. Test methods for evaluating solid waste: physical/chemical methods. SW-846. Washington, D. C.: USEPA, Office of Solid Waste and Emergency Response, 1986.

[7] APHA. Standard methods for the examination of water and wastewater (20th ed.). Washington, DC: American Public Health, 1995.

[8] El-Hissy, F.T and Khallil, A.M. Studies on aquatic fungi in Delta region (Egypt). Zentralbl Microbiol, 144:421-432, 1989.

[9] Coker, W. C. The Saprolegniaceae, with notes on other water molds. University of North Carolina Press, Chapel Hill, 1923.

[10] Johnson, T. W. The genus *Achlya*: Morphology and Taxonomy, p 188, University of Michigan, Ann Arbor, 1956.

[11] Sparrow, F.K. Jr. Aquatic phycomycetes. Univ. of Michigan Press. Ann Arbor. Second Ed, 25:1187, 1960.

[12] Waterhouse, G. M. Key to *Pythium* Pringsheim. Mycol Inst Kew, Surrey, England, pp 1-15, 1967.

[13] Waterhouse, G. M. The genus *Pythium* pringsheim. Mycol Pap Commonw Mycol Inst, Kew, Surrey, England, pp 1-71, 1968.

[14] Seymour, R.L. The genus *Saprolegnia*. Verlag Von J Cramer, Germany, p 124, 1970.

[15] Raper, K.B. and Thorn, C. A manual of the penicillia. Williams & Wilkins Co., Baltimore, 1949.

[16] Pitt, J. I. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic press, 1980.

[17] Raper, K. B. and Fennell, D. I. The genus *Aspergillus.* Williams & Wilkins Co., Baltimore, 1965.

[18] Booth, C. *Fusarium*, laboratory guide to the identification of the major species. Commonwealth Mycological Institute, Kew, Surrey, England, 1971.

[19] Arx, J. Avon. Genera of Fungi Sporulating in Pure Culture. 3rd Edition. J. Cramer, Vaduz, 1981

[20] Domsch, K. H., Gams, W. and Anderson, Traute-Heidi. Compendium of soil fungi, Vol. I & II. Academic Press, 1980.

[21] Watanabe,T. Pictorial Atlas of Soil and Seed Fungi. Morphologies of Cultured Fungi and Key to Species. 2nd edn. CRC Press, Boca Raton. 2002.

[22] Arx, J. Avon., Guarro, J. and Figueras, M. J. The Ascomycete genus *Chaetomium.* Beih. Nova Hedwig*ia* 84: 1-162, 1986.

[23] Kirk, P.M., Cannon, P.F., David, J.C., and Stalpers, J. A. (Eds.). Ainsworth & Bisby's Dictionary of the Fungi, 9th Edition. (CABI Publishing), 2001.

[24] Greenberg, A., Clescerl, L. and Eaton, A. Standard methods for the examination of water and wastewater. 18th ed. Am. Public Health Assoc., Washington, DC, 1992.

[25] MacFaddin, J.F. Media for isolation, cultivation, identification and maintenance of medical bacteria. Williams & Wilkins, Baltimore/London, 1985.

[26] Fawcett, J.K. and Scott, J. Determination of blood urea using the berthelot reaction. J. Clin. Path., 13:156-162, 1960.

[27] Seeling, F. and Wust, T. Modified methodology determination of creatinine. Z. Ernaehryg Swiss., 2(4): 169-176, 1969.

[28] Barham, D. and Trinder, P. The determination of uric acid in serum Aralyst., 97: 142-145, 1972.

[29] Wallnofer, H., Schmidt, E. and Schmidt, F. W. Kinetic method for determination of ALT activity according recommendations of the expert panel of the International Federation of Clinical Chemistry (IFCC)". Gerog Thieme Verlag, Stuttgart, 1974.

[30] Tietz, N. W. Clinical guide to laboratory tests. 3rd ed., Philadephia, Pa: WB Saunders, pp: 76-77, 1995.

[31] Persijin, J. P. and Van der Slik, W. A new method for determination of GGT. J. clin. Chem. Clin. Biochem. 4: 421, 1976.

[32] Barnett, R. N., Skodon, S. B. and Goldberg, M. H. Performance of kits used for clinical chemistry analysis of calcium in serum. Am. J. Clin. Path., 59:836-845., 1973.

[33] Goldenberg, I. I. and Fernands, A. Simplified method for the estimation of inorganic phosphorus in body fluids Clin. Chem., 12: 871, 1966.

[34] Bregnhøj, H. Instruction Manual for Determination of Fluoride in Water Samples and Instruction Manual for Determination of Total Alkalinity (TAL). Centre for Developing Countries, Technical University of Denmark, 1992.

[35] Browne, D.; Whelton, H. and Denis O'Mullane, D. Fluoride metabolism and fluorosis, Journal of Dentistry, 33; 177-186, 2005.

[36] Snedecor, G. W. and Cochran, W. G. Statistical Methods. 7th ed., English Language Book Society, London, 1980.

[37] Anonymous, A. Cohort Software Corp. Costate User Manual Version 3.03, Barkley Ca, USA, 1989.

[38] Saleh, M. (2008). Design and establishment of a purification unit on El-Zayitona Well in Saint Katherine, South Sinai, Egypt (www.saintkatherinecenter.org)

[39] Dahi, E., Singano, J.J. and Nielsen, J.M (1995), "Kinetics of defluoridation of water by Calcite Magnesia and clay". Proceedings of the first International Workshop on Fluorosis and Defluoridation of water. Published by the International Society of Fluoride Research Editorial Office, pp 58-66, 1995.

[40] Diab, A. Bacterial survey of well waters of Wadi El-Sheikh in south of Sinai. M.Sc. Thesis, Suez Canal University, Ismailia, Egypt, 1984.

[41] WHO. Draft guidelines for safe recreational-water environments: Coastal and fresh water, draft for consultation, Geneva, October 1998, World Health Organization (EOS/DRAFT/98.14), Geneva, 207 pages, 1998.

[42] Keswick, B. H. Sources of groundwater pollution. In: Ground water pollution microbiology (Bitton G & Gerba CP, Eds.). John Wiley & Sons, Inc., New York. pp. 39-64, 1984.

[43] Entry, J. and Farmer, N. Ground water quality: Movement of coliform bacteria and nutrients in ground water flowing through basalt and sand aquifers. Journal of Environmental quality 30: 1533-1539., 2001.

[44] WHO. Guidelines for Drinking-water Quality. Third edition. Volume 1 Recommendations. Geneva, 515 p, 2004.

[45] Ward, M. H., Mark, S. D., Kantor, K. P., Wesisenburger, D. D., Correavillasenor, A. and Zahm, S. H. Drinking water nitrate and the risk of non-Hodgkin's lymphoma. Epidemiology 7: 465-471., 1996.

[46] Shendi, E. H. and Geriesh, M. H. Landfills as a tool for controlling the environmental pollution of the natural protectorates (Saint Catherine area as a case study. Proceedings of the 5th conference on Geology and Sinai Development, Saint Katherine: 65-81, 1998.

[47] Nriagu, J. O. and J. M. Pacyna. Quantitative assessment of worldwide contamination of the air, water and soils with trace metals. Nature (London), 333: 134-139, 1988.

[48] G.M. Gadd and C. White, "Microbial Treatment of Metal Pollution--A Working Biotechnology?," Trends Biotechnol., 11:353-359,1993.

[49] Babu, M. N. S., Somashekar, R. K., Kumar, S. A., Shivanna, K., Krishnamurthy, V. and Eappen, K. P. Concentration of uranium levels in groundwater. Int. J. Environ. Sci. Tech., 5 (2), 263-266, 2008.

[50] Ghodeif, K. and El-Shafey, M. High-yield groundwater bearing fractures in crystalline rocks: A case study from Saint Katherine area, South Sinai, Egypt. In: 3rd International Conference on the Geology of Africa, in press, 2003.

[51] Susan Lillard. Mycotoxin list. http://www.mold-help.org/content/view/776/ (accessed January 13, 2009), 2008.

[52] Schiffl, H. Fluoridation of drinking water and chronic kidney disease: absence of evidence is not evidence of absence. Nephrology Dialysis Transplantation, 23:411, 2008.

[53] Bansal, R. and Tiwari, S. C. Back pain in chronic renal failure. Nephrology Dialysis Transplantation, 21:2331-2332, 2006.

[54] Arnala, I. Effects of fluoride on bone in Finland. Histomorphometry of cadaver bone from low and high fluoride areas. Acta Orthop Scand. 56(2):161-6, 1985.

[55] Paul, C. E. and Larry, W. Analytical research lab, Phoenix, Arizona 85021 USA, 1990.

[56] Julian J. C., and O'Hara, J. Lead Absorption in Children", Urban & Schwarzenberg, Baltimore-Munich, 1982.

[57] Forsman, B. Dental fluorosis and caries in high fluoride districts in Sweden. Community Dentistry and Oral Epidemiology 6:132–48, 1974.

[58] Opinya, G. N., Valderhaug, J., Birkeland, J. M., Lokken, P. Fluorosis of deciduous teeth and first permanent molars in a rural Kenyan community. *Acta Odontologica Scandinavia* 49: 197–202, 1991.