Evaluation of Some Physico-chemical and Detection of Parasites in Sediment of Hamrin Lake

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Abstract

Samples from the clay of the Hamrin lake were taken to detect its composition and the presence of different types of parasites that affect the workers' health with clays.

The concentrations of metal major oxides ranges (48.4-51.2%) for SiO_2, (25.2-28.1%) for Al_2O_3 and (13.5-14.1%) for Fe_2O_3, while the concentrations of metal minor oxides were relatively low ranges (1.9-2.5%) for K_2O and less than 0.8% for (Cr_2O_3, CuO, K_2O and Na_2O. A non-detectable percentage for MgO, MnO, P_2O_3, TiO_3 and ZnO were obtained.

The most abundant clay in the samples was Kaolinite due to X-ray diffraction patterns, while quartz was the most abundant non-clay mineral.

This research detects cysts and trophozoites from several protozoa parasites in the soil such as (*Entamoeba* spp., *Giardia* spp., *Cryptosporidium* spp., *Balantidium* spp.; *Cyclospora* spp), as well as eggs of *Ascaris* and *Ancylostoma* spp.

Key Words: Hamrin lake, Clay, Parasites, Oxides and X-ray Diffraction.

تقييم بعض الخواص الفيزيائية–الكيميائية الكشف عن الطفيليات في طمى بحيرة حمرين

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الخلاصة

اخذت عينات من تربة بحيرة حمرين لدراسة تركيبها وللكشف عن وجود أنواع مختلفة من طفيليات الابتدائيات التي تؤثر على صحة العاملين مع الصلصال بطرائق مختلفة. تراوحت نسب تراكيز الأكاسيد الرئيسة من (48.4–51.2%) لـ SiO_2، و(25.2–28.1%) لـ Al_2O_3 و(13.5–14.1%) لـ Fe_2O_3 ، في حين كانت تراكيز الأكاسيد الثانوية منخفضة نسبياً وتتراوح ما بين (1.9–2.5%) بالنسبة لاوكسيد البوتاسيوم، وأقل من 8% لجميع الاكاسيد الاخرى، بينما كانت النسب المئوية لاكاسيد المغنيسيوم والفوسفات والتيتانيوم والخارصين منخفضة جداً لدرجة الاهمال . بينت انماط حيود الأشعة السينية ان الكولنيت كان المكون الاوفر للصلصال، بينما كان الكوارتز المكون الاكثر وفرة من غير الصلصال.

بين البحث ان بيوض طفيليات الابتدائيات هي الاكثر انتشاراً مثل بويضات الانتميبا، والجيارديا، والكريبتوسبوريديم، والبلانتيديوم، والخراجات القولونية (سايكلوسبورا)، كما وجدت بيوض ديدان الإسكارس والانكلستوما.

الكلمات المفتاحية: بحيرة حمرين، صلصال، طفيليات ، أكاسيد و حيود الاشعة السينية.

Introduction

The lake of dam Hamrin in Iraq is located on Diyala River. The lake was 2040 Km^2 in 1981, but was reduced to 20% of its original size after Iran built a dam on the Al-Wand River in 2007 (Central Statistics Office, 2013).

A small town was built illegally on the dry surface of the lake and building contractors used the surface of the lake to obtain clays and other building materials (Mulder *et al*., 2015).

Several studies were done concering the topography of the area, but all these studies were aimed to study the composition of the clay in the sediment (Clay is a finely-grained natural rock soil material that combines one or more minerals with possible traces of quartz (SiO_2), metal oxides (such as Al_2O_3, MgO etc.) and organic matter) in that area.

It is aimed also to survey types of protozoa parasites from the dry land of the lake that affects the workers' health in different ways (Moshfe *et al*., 2000, Keserue *et al*., 2011).

Cats, dogs, sheep and cows are excellent carriers for many protozoan parasites (such as *Giardia*, *Cryptosporedium* and *Cyclospora*) as well as zoonotic helminthes parasites, such as *Ascaris, Ancylostoma, Toxocara, Trichuris vulpis* and *Spirometra* species (Hotez *et al*., 2008, Taherian, 2009, Beiromvand *et al*., 2013, Jeandron *et al*., 2014, Bilung *et al*., 2017).

The defecating and scavenging rubbish by these animals can lead to the contamination the soil within their roaming territories.

Aims of the study

The study aims by detecting the concentrations of major and minor oxides and their other properties in the sediment, as well as to detect the presence of any parasites that affects the workers' health in the area.

Materials and Methods

Area Study

Lake Hamrin is located in the Diyala Governate, Iraq, and its water come from the Diyala River which is divided into several branches before ending in the lake.

A small town was built, illegally, on the shores of the lake and 20,000 inhabitants live there for the past 23 years under poverty line.

Samples Collecting

The lake's land consists of several layers of alternated red-gray clays and sand stones.

Samples of clay were taken from four different moisture places near the shores of the lake in three successive winters (2014-2017). The leaves and debris on the surface of the soil were removed and approximately 200 to 250 g of soil was scrapped off the surface (to 1 cm depth) and collected in labeled plastic bags.

The samples that were used for detection of parasites were collected every other day (for 10 days), crushed, sieved out (using 0.150mm sieve) and preserved with sterile phosphate-buffered saline (PBS) (150 mM NaCl, 15 mM KH_2PO_4, 20 mM Na_2HPO_4, 27 mM KCl, pH 7.4;) with 2.5% formalin at 4°C and then they were transported to the laboratory on the same day and processed immediately, or stored at 4°C for further microscopic observation (Gaspard & Schwartzbrod, 2003; Garcia, 2007).

Physical Analysis

Oxides Concentration Study

The percentage of major metal and minor metal oxides concentrations in the clay were determined by X-ray diffracting patterns of the sample by using X-ray diffractometer (XRD) (Empyrean, PANalytical, Netherlands). (Ealick, 2000, Thangadurai *et al*., 2005, Hammond, 2009).

Particle Size Distribution

The clay samples were crushed and sieved out using 0.150mm sieve and then they were divided into 100g lots. Each lot was washed with 100ml 5% $(NaPO_3)_6$ (as a deflocculant) and mixed thoroughly with 250ml of distilled water, and then left to stand for 40 min and was completed to 1000 ml.

The dimensions of a particle are measured using laser diffraction spectroscopy (Mastersizer 3000E, Malvern Instruments, England).

Determination of Particle Density

The particle density was measured according to the method of Cresswell and Hamilton (2002).

Each sample was heated for 20 h at 110°C, but –in this case- each sample must be weighted (and its diameter was measured) before heating and after it was cooled down.

Determination of Porosity

The surface porosity (ϕ) of a sample was calculated from the particle density according the method of Horgan (1998).

The Determination of Soil Moisture

Estimated soil moisture was measured using 10ml soil moisture sensor (VH440, Sigma) after calibration.

Determination of Organic Carbon Content

The soil carbon content was measured according to colorimetric method (Nelson and Sommers, 1996).

10g of clay were crushed and sieved out using 0.150 mm sieve and then mixed with 100ml $K_2Cr_2O_7$.

Concentrated H_2SO_4 (200ml) was added to the mixture with careful shaking before the mixture left to stand for 1h at room temperature.

10 ml of sodium dichromate (0.5N) mixed with 4M H_2SO_4 was added to 1g of clay (after it was crushed and sieved out as before). The mixture was shaked for 20min and then left to stand for 1h and the upper level of the mixture was removed carefully and its absorption was read at 660nm using vis-uv spectrophotometer (Shimadzu, Japan).

Total Nitrogen Content

This was carried out by using the modified classical Kjeldahl method (Kjeldahl, 1883, Jaber *et al*., 2000).

Phosphorus Content

This was determined according to Bray and Kurtz method (1945).

One gm of the sample was crushed and sieved out before mixing with ammonium fluoride (15ml) and HCl (25ml). After shaking, distilled water was added to 500ml, and after another shaking, the mixture was centrifuged and the supernatant (4ml) was added to 6ml L-ascorbic acid in distilled water and 4ml ammonium molybdate solution.

The mixture stand for 5min, and absorbance at 852nm was read in vis-uv spectrophotometer (Shimadzu, Japan). A standard phosphorus solution (0-50mg/l) was prepared.

Detection of Soil Parasites

Microscopic Observation

Freshwater protozoa were collected and classified according to Finlay and Esteban (1998), while helminthes were collected and classified according to Sepulveda and Kinsella method (2013).

Each collected sample was put into a clean 15 ml test tube and mixed vigorously with 3ml Ether for 1 to 2 min and then centrifuged for 5 min at 2,000 rpm.

The centrifugation resulted in four layers comprising the top: ether, a debris plug, formalin and lastly the sediment-containing parasites at the bottom. The supernatant was decanted. A drop of the pellet was examined on a slide (with or without mixing with Lugol’s Iodine solution) under a light compound microscope to detect and identify cysts, oocysts or helminthes eggs. They were recorded to their specific genus. At least 30-40 slides were examined (Casemore *et al*., 1983, Downes and Ito, 2001, Garcia, 2007).

The pellet was stained according to modified Zeihl–Neelsen and modified trichrome to detect protozoa parasitic cysts and oocysts.In some case, and before examining the cysts or eggs, the samples were sonicated for few minutes (3-6 min) in an ultrasonic bath (TUC-600; M. Scherrer AG, Wil, Switzerland) at 35 kHz and 600 W in order to dissociate the majority of the agglomerated cysts.

Flotation Technique

The eggs were isolated by flotation technique through mixing and sieving 4g of soil with 26ml of flotation solution (3.36g zinc chloride in 100ml of distilled water). Few drops of the mixture (after standing for 30 seconds) were taken and observed by microscope (Charitha *et al.*, 2013).

Results and Discussions

Chemical Composition of the Clays

The major and minor concentrations were calculated through XRD. The major metal oxides' concentrations are ranging from 48.4 to 51.2% for SiO_2, from 25.2 to 28.1% for Al_2O_3 and from 13.5 to 14.1%) for Fe_2O_3 (Table 1).

The minor metal oxides' concentrations were much lower and ranging from 1.9 to 2.5% for K_2O and less than 0.8 for all other clay samples, while the percentage for MgO, P_2O_3, TiO_3 and ZnO were non-detectable (Table 2). Quartz was considered to be the most non-clay material according to X-ray diffraction, and closely corresponded with SiO_2 values, while the most dominant clay was kaolinite in the samples. The muscovite and bentonnite were observed in very few samples.

Table (1) Major Oxides (%) in Clay Samples

	SiO_2	Al_2O_3	Fe_2O_3		Total
A	51.2	26.7	13.5		91.4
B	49.3	25.2	13.6		88.1
C	48.9	27.1	14.1		90.1
D	48.4	28.1	13.9		90.4

Table (2) Minor Oxides (%) in Clay Samples

	CaO	Cr_2O_3	CuO	K_2O	MgO	MnO	Na_2O	P_2O_3	TiO_2	ZnO		Total
A	0.1	0.1	0.33	2.5	n.d	0.1	0.1	n.d	n.d	n.d		3.23
B	0.1	0.1	0.35	2.0	n.d	0.1	0.1	n.d	n.d	n.d		2.75
C	0.2	0.2	0.32	2.5	n.d	0.2	0.2	n.d	n.d	n.d		3.62
D	0.2	0.2	0.31	1.9	n.d	0.2	0.2	n.d	n.d	n.d		3.01

n.d. non detectable

Moisture Content

The availability of water regulates most biological and chemical activities in soil, so any changes in this availability will influence soil organisms. This influence of availability may take complex interactions either with nutrients, temperature, or the distribution of pore sizes of the soil (Schnurer *et al.,* 1986).

Cysts and oocycts of parasites require water to their lives, but they can tolerate certain amount of dryness tolerance varies among them, so moisture content was monitored in clay during the experiments.

The gradual decrease of the moisture will eventually reduce the porosity of the clay which makes it more compact and decrease the amount of oxygen present (Sandee *et al*., 2015).

These living organisms decompose proteins and simple sugars easily, while take long time to decompose polysaccharides or lignin (Alum *et al*., 2014).

Protozoa and Helminthes Recovery Rate

Our study found intestinal protozoa cysts and trophozoites of (*Cryptospordium*, *Cyclospora*, *Entamoeba* and *Giardia* spp.) in the soil.

Eggs from *Ascaris* and *Ancylostoma* spp. were detected in the soil, but other helminthes eggs (such as of *Taenia, Trichuris* spp) were not detected (Alum *et al.,* 2014).

The trophozoites-in general- were difficult to observe due to their sensitivity and their ability to degenerate rapidly (unlike cysts).

Cryptosporidium spp. oocysts were stained as spherical pinkish red, while *Cyclospora* spp. oocysts were screened as light clear pink to deep red, containing granules.

Giardia spp. cysts and trophozoites were shown as very light red under microscope (Rajurkar *et al.,* 2012). *Entamoeba* spp. cysts and trophozoites were yellowish red when stained with Lugol's iodide, while *Balantidium* spp. trophozoites were stained brown red and its cysts stained with lighter color with the same stain.

Other studies in similar areas claimed the prevalence of such parasites due to several reasons such as poor diet, poor sanitation, poor hygiene, poor medical care and so on (Daryani *et al*., 2008, Taherian, 2009, Al-Mergin, 2010, Kotloff, 2013, Sandee *et al*., 2015).

Lake Hamrin area is a contaminated area due to several factors such as the presence of waste water that flows into the river and be used for irrigation.

The debris of cars, trucks and machines that were used for extracting the clays.

Contamination of soil with animal wastes produced by so many stray dogs and cats, as well as sheep and cows in the area contribute significantly to the transmission of such parasites.

Increased application of improperly composed manures to soil as fertilizers increases parasite contamination (Daryani *et al.,* 2008*).*

Most farmers in the area irrigated their vegetables with contaminated water which is responsible for the increase of pathogenic parasites., Daryani *et al*,. 2008, Al-Mergin, 2010,).

The most prevalent parasites detected were helminthes (31.2%), *Giardia* spp. (25.4%), *Entamoeba* spp. (17.7%), *Balantidium* spp. (14.0%), and *Cryptosporidium* and *Cyclospora* spp. (11.7%) (Fig. 1).

Table (3) Number of Protozoan Oocysts and Cysts and Helminthes' Eggs Found in the Four Sites of the Experiments and Their Total Percentage.

Parasites	Sites of the Experiments				Total no.	%
	A	B	C	D		
Giardia	88	97	86	102	373	25.4
Entamoeba	48	53	62	43	206	14.0
Cryptosporidia and *cyclospora*	36	51	34	51	172	11.7
Balantidium	56	81	63	60	260	17.7
Helminthes eggs	150	70	142	97	459	31.2
					1470	

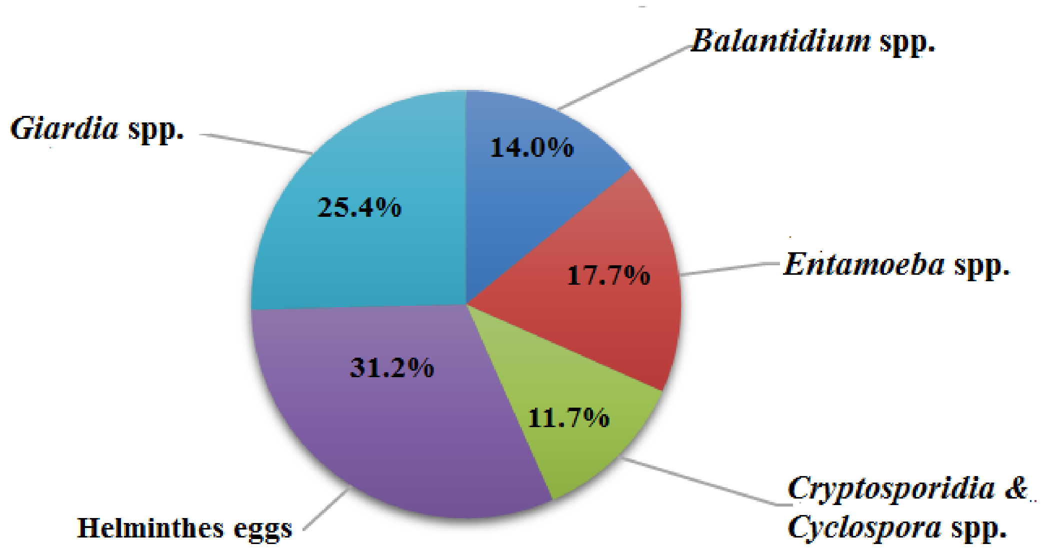

Fig. (1) the Recovered Oocysts,Cysts or Egge of Protozoa or Helminthes.

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