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RESEARCH ARTICLE

DETERMINE OF HEAVY METALS CONCENTRATIONS BY USING SCANNING ELECTRON MICROSCOPY AND ENUMERATE OF BACTERIA IN AL-DALMAJ MARSH

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ABSTRACT

The current study included determining some Heavy metals in water on Al-Dalmaj marsh south of Iraq by use scanning electron microscopycoupled with energy dispersive spectrometer(SEM-EDS) **.**Samples were collected in two seasons winter and summer for water. The chemical studied parameter such pH, and heavy metals concentrations , which indicate polluted as it . The results showed increased of heavy metal concentrations comparative with WHO concentrations whereas: Mn:4.2584-3.334 , Cd : 0.276226-0.16226 , Ni : 1.0349- 0.5328 , Pb : 1.2765-0.8305 , Co : 1.0183-0.6176 , Cu : 1.9787-1.7746 , Zn : 11.789-9.2054 , Hg : 0.6789-0.2434 µg/ml, respectively in two seasons Sumer and winter. The range of pH was 7.9-8.8 during the winter, while in summer it was slightly acidic to alkaline (6.6-7.4).The results of enumerate bacteria of plate counts on media MacConkey agar and EMB agar gave much higher growth to various species on EMB agar media for 1,2,4 and 5 from the sites, comparative between MacConkey ager media .

INTRODUCTION

Trace metal contaminations are important due to their potential toxicity for the environment and human beings (22). Some of the metals like Cu, Fe,Mn, Ni and Zn are essential as micronutrients for the life processes in animals and plants while many other metals such as Cd, Cr, Pb and Co have no known physiological activities(3). Metals are non-degradable and can accumulate in the human body system, causing damage to nervous system and internal organs (17). However, the rivers play a major role in assimilation or transporting municipal and industrial wastewater and runoff from agricultural and mining land (18).

Heavy metals are sometimes called “trace elements”. They are the metallic elements .Heavy metals have become of particular interest in recent decades within the framework of environmental investigation. This has without doubt been due to the fact that highly sensitive analytical procedures are available for determining and detecting metal content with high precision. Medical geology is a subfield of geology that studies the effects of chemical in the environment, especially trace elements, on the health of humans and animals. The contribution of the geology is to help isolate aspect environments that may influence the incidence of disease. The ultimate source of the body trace elements is generally rocks. The concentration of trace elements in rocks is varying by rock type. Sometimes, they become concentrated in soil, water, or in air taken up by plants and ingested by humans or animals. Pure water does not exist in nature. The contamination of water is directly related to the degree of contamination of our environment. Rainwater collects impurities while passing through the air. Streams and rivers collect impurities from surface run off and through the discharge of sewage and industrial effluents; these are carried to the rivers, lakes or reservoirs that supply our drinking water (19).

The present study aimed to determine of heavy metal concentrations in various seasons by use electron microscopic scanner in Al-Dalmaj marsh water and pH values. The research work was carried during summer (May, 2014) and winter (January, 2014).

Site Description

The study area was described in previous work[15].

MATERIALAND METHODS

In order to achieve the research objective, samples were collected from five different locations of Al-Dalmaj marsh to evaluate the heavy metal contamination during various seasons(summer and winter). Criteria for selection of sampling station were based on the locations of use pattern to quantify

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heavy metal concentration. Five sites were selected from Al-Dalmaj marsh to collected samples .The Samples were taken from 10 to 15 cm below the water surface using acid washed plastic container to avoid unpredictable changes in characteristic as per standard procedures (8). Samples were collected in May 2014 for summer season, and in January 2014 samples were collected for winter season. Care was taken to collect subsequent samples from same location in both the seasons.

The metal concentrations on the samples were assured as to fulfill the criteria of the limit of detection of the following microscopic analysis by applying total digestion to the samples and analyses in electron microscopic unit in kufa university. Previously freeze dried samples were grounded on agate mortar. Bulk samples, samples <50 micrometer and magnetic samples (samples separated magnetically) were dispersed in alcohol and filtered on a 0.5 µm nucleopore carbonate filter and posed on a carbon sample holder with a diameter of about 1 cm. The amount of the sample dispersed is approximately 100-200 µg. Prepared filter was checked under microscope to confirm that the particles were evenly dispersed to facilitate the microscopic analysis that follows. Finally, a thin layer of carbon was coated on the filter to allow high electron conductivity during analysis. Samples were analyzed using a Scanning Electron Microscope (SEM) JEOL JSM 840 coupled with an X-Ray microanalysis system from Princeton Gamma Tech (PGT). A ray of generated electrons of the size of a few angstroms is scanned over a randomly located field. A numerical image is produced from the backscattered electron beam with particles ranging from 0.2 to 20 µm. X-ray spectrum generated from these particles are then acquired (Figure 1) using a high purity Germanium (HPGe) detector and digital pulse processing from PGT. Tens of spectrums of particles per sample were analyzed automatically to assure that results are qualitatively representative.

The spectrum is analyzed by the energy dispersive spectrometer (EDS) and then classed according to the pre-defined sediment, as well as metal phases. Predefined sediment phases included various heavy metals. Heavy metals were chosen according to the capability of the SEM-EDS to identify particles and analyze spectra. Organic matter and amorphous elements are not defined since these phases cannot be detected by the SEM. On the other hand, metal classes consisted of Mn, Cd, Ni, pb, Co , Cu , Zn and Hg. These metals were chosen due to their high quantity which allows them to be detected on the spectrometer.

A manual check was done by randomly observing spectra and particles to confirm that they were correctly classed. Interesting particles was further analyzed by producing morphological images and elements in marsh water.

Statistical analysis

Least significance deviation (L.S.D) test were used to evaluate the significant difference in the concentration of different studied metals with comparative between season and WHO concentrations. A probability at level of 0.05 or less was considered significant (10).

RESULTS

Determination of heavy metals levels in Al-Dalmaj marsh water by use electron microscope examination, pH value and enumerate of polluted bacteria:

According to the results of the detected heavy metals levels in all collected samples of Al-Dalmaj marsh water it's clear to see among all the water samples numbers which were collected, the huge pollution Values of all the currently heavy metals in the marsh comparison of WHO concentrations and two seasons as shown in figure 2 and 3.The results showed of significant difference between average of heavy metal concentrations and WHO concentrations as following:

Mn:4.38078-3.51306 , Cd : 0.294024-0.193012 , Ni : 1.52496-0.67814 , Pb : 1.62814-0.79728 , Co : 1.26898-0.74614 , Cu : 2.04272- 1.40324 , Zn : 12.005 - 9.16214 , Hg :0.14066 - 0.01968 µg/ml, respectively in two seasons Sumer and winter (figure 1 and 2).

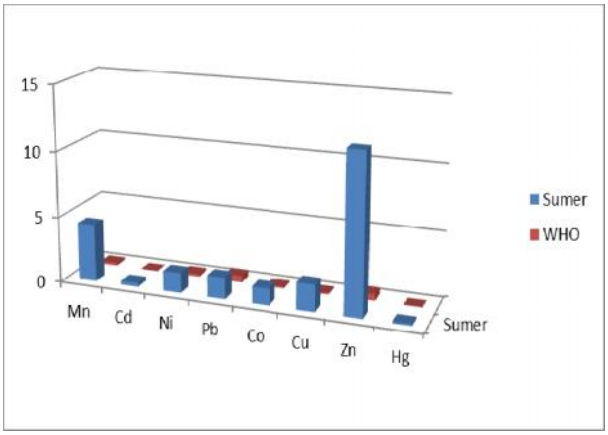


Figure 1 Concentrations of heavy metal in water samples from Al-Dalmaj marsh at Summer season comparative between WHO concentrations .

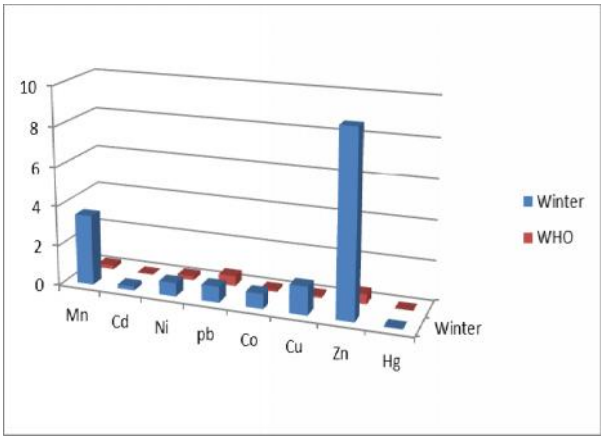


Figure 2 Concentrations of heavy metal in water samples from Al-Dalmaj marsh at winter season comparative between WHO concentrations .

The results revealed that in most of the sampling stations, water was slightly alkaline except at very few stations in Al-Delmaj marsh where the water was found to be slightly acidic. The range of pH was 7.9-8.8 during the winter, while in summer it was slightly acidic to alkaline (6.6-7.4).

Marsh water samples used to enumerate bacteria.Table 6 summarizes the results of plate counts on media MacConkey agar and EMB agar. The marsh water collected from five sites was used to enumerate of polluted bacteria .Water samples gave much higher growth to various species on EMB agar media for 1,2,4 and 5 from the sites, comparative between MacConkey ager media .

Tables 1 Heavy metals concentrations in samples collected from five stations in Al-Dalmajmarsh water and comparative between WHO concentrationsin summer.

Summer season	Mn	Cd	Ni	pb	Co	Cu	Zn	Hg
S1	4.2584	0.27622	1.0349	1.2765	1.0183	1.9787	11.789	0.1022
S2	3.9623	0.2951	1.8765	1.9456	1.3456	1.9899	12.2314	0.1552
S3	4.5561	0.3013	1.7945	1.8976	1.2567	2.1345	11.9987	0.1905
S4	4.4845	0.3108	1.2345	1.6785	1.2567	1.8990	12.1123	0.0987
S5	4.6426	0.2867	1.6844	1.3425	1.4676	2.2115	11.8936	0.1567
Average	4.38078	0.294024	1.52496	1.62814	1.26898	2.04272	12.005	0.14066
WHO	0.2	0.01	0.2	0.5	0.1	0.1	0.5	0.001
L.S.D	0.1	0.002	0.01	0.03	0.04	0.02	0.05	0.002

Tables 2 Heavy metals concentrations in samples collected from five stations in Al-Dalmajmarsh water and comparative between WHO concentrations in winter.

Winter season	Mn	Cd	Ni	pb	Co	Cu	Zn	Hg
S1	3.526	0.16226	0.5328	0.8305	0.6176	1.3746	9.2054	0.0234
S2	3.3288	0.1933	0.5478	0.7653	0.7568	1.1976	9.4467	0.0345
S3	3.4678	0.2043	0.6789	0.8574	0.8765	1.5435	8.8790	0.0178
S4	3.6784	0.2145	0.6745	0.6546	0.6899	1.2110	9.2234	0.0104
S5	3.5643	0.1907	0.9567	0.8786	0.7899	1.6895	9.0562	0.0123
Average	3.51306	0.193012	0.67814	0.79728	0.74614	1.40324	9.16214	0.01968
WHO	0.2	0.01	0.2	0.5	0.1	0.1	0.5	0.001
L.S.D	0.2	0.04	0.06	0.03	0.02	0.06	0.4	0.001

Table 3 Enumerate of polluted bacteria in marsh water sampleson media MacConkey agar and EMB agar

Samples	Enumerate total bacteria on MacConkey agar	Numerate total bacteria on EMB agar
S1	$17*10^5$	$47*10^5$
S2	$56*10^5$	$130*10^5$
S3	$8*10^5$	-
S4	$47*10^5$	$122*10^5$
S5	$27*10^5$	$93*10^5$

DISCUSSION

Scanning electron microscopy proves to be a powerful method in measuring to heavy metals concentrations and its carrier phase. Particle analyses of Al-Dalmaj marsh water are shown to be effective in determining the sources of contamination. Its application in the future will be used in understanding monthly, weekly, and even daily dissolved metal variability in Al-Dalmajmarsh water. The comparing of heavy metals levels determined values in Al-DalmajmarshWith WHO concentrations , reflects the huge pollution values of all the currently heavy metals levels in the marsh. These results came along with others which were carried by (4) indicating that all heavy metals levels, levels where higher than WHO concentrations in Iraqi marshes, while it disagree with other results carried by(7) in the same area of marshes. Increasing of heavy metals levels in the entire world may come by manyreasons including untreated sewages and waste water which were thrown in river or other water bodies, in addition to other natural sources (9)(2) increased population in the last few decades have caused a dramatic increase in the demand for river water, as well as significant deteriorations in water quality throughout the world (4).There are two ways of lead entrance into the aquatic environments of southern Iraqi marshes;natural And anthropogenic ways(5). Natural ways include storm dust fall, erosion or crustal weathering and decomposition of the biota in the water, without mention to other ways such as lava and volcanic projectiles or acid rain (4).on the other hand, anthropogenic ways can be assembled by untreated domestic and industrial sewage which were discharging to the Tigris and Euphrates Rivers then reaching to southern marshes or by discharging directly into marshes (7), the wars that happened in that area also served as a source of heavy metals due to using the extensive burning, heavy bombing and shelling, and widespread use of chemical weapons (4).In addition to all these reasons, it is possible to believe that metals in the study area were derived mainly from the igneous mineral deposits in the Iraq-Iran Mountain range (11).

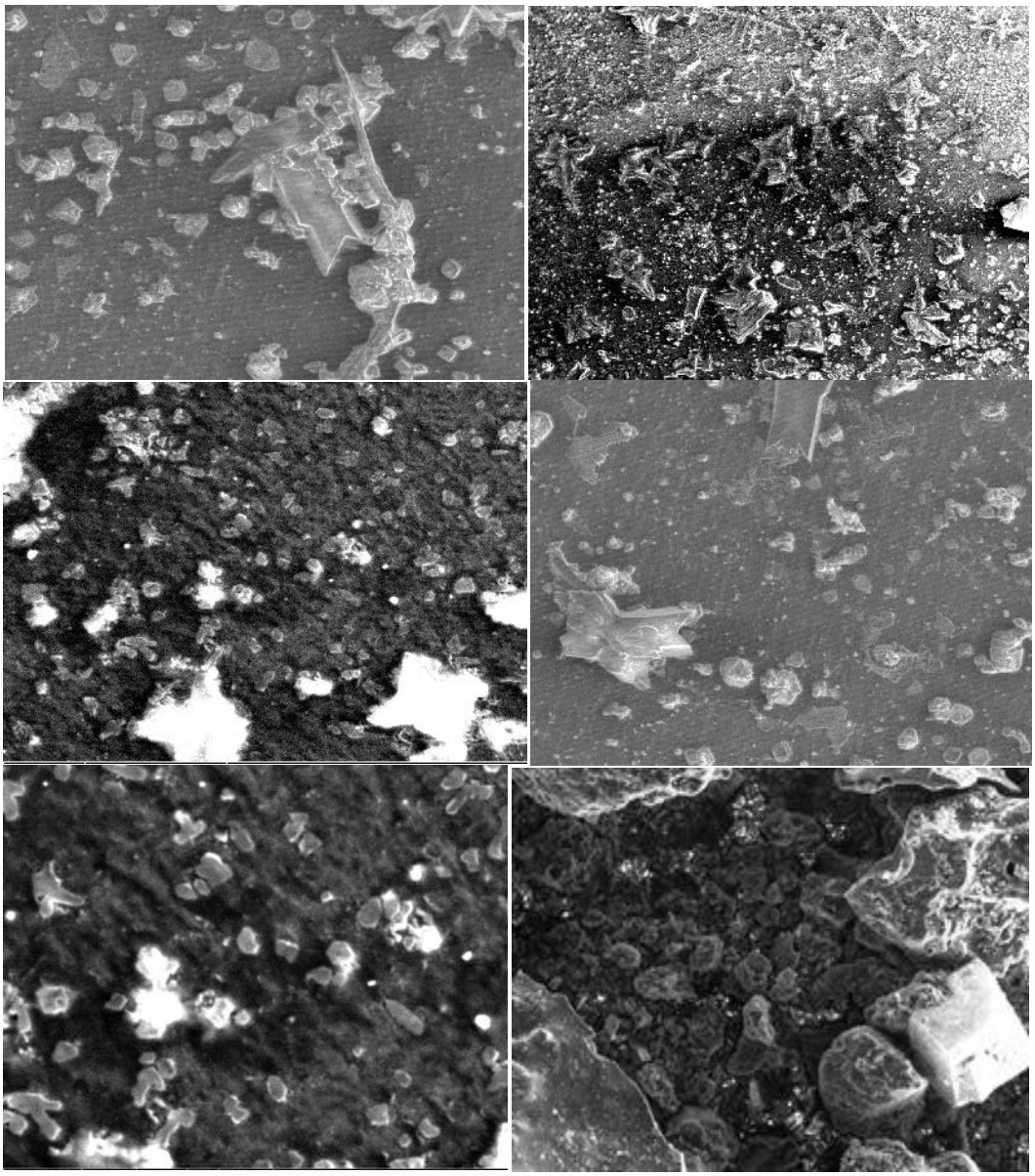
Al-Juboury recorded a slight pollution of heavy metal in the up regime of Tigris in the north of Iraq, he suggested that the clay and heavy minerals may form an important source for the natural pollution by the heavy metals in the recent sediments of the Tigris River, as well as most of the pollution came from the wastewater contributed to the river (6).

The concentrations of heavy metals during the winter closure period were lower than those after the winter period (Table 2), because of the increased consumption of this element by the phytoplankton (14). Low heavy metals concentrations in the surface water may be related to the contribution of phytoplankton, pH and dissolved oxygen concentrations. The negative correlation between heavy metals such as Mg and Zn concentrations is may be a result of precipitation of the elements as hydrous metal oxides (13). The permissible levels of heavy metals in water used for domestic purposes are quite low and in water for continuous irrigation the maximum acceptable concentrations (21). The major sources of heavy metals are the domestic wastes, municipal wastes followed by dumping and atmospheric deposition (16).

In brief, heavy metal contamination in the environment has become a serious problem due to the increase in the addition of these metals to the environment. Natural sources as well as the anthropogenic sources account for this contamination, which has become a threat to public health. Heavy metals are being released to the environment (1). Contamination of the aquatic environment by toxic metal ions is a serious pollution problem. Due to the fact that, unlike organic pollutants, chemical or biological processes can't degrade toxic metal ions, to remediate the aquatic environment, the toxic metal ions should be concentrated in a form that can be extracted conveniently, possible for reuse or at least for proper disposal. Natural resources including plants and microorganisms are extensively explored to combat metal ion pollution (12).

years due to air pollution, water pollution, and hazards over uses of pesticides in agriculture. Trace amounts of metals are common in water, and these are normally not harmful to our health. In fact, some metals are essential to sustain life. Calcium, magnesium, potassium, and sodium must be present for normal body functions. Cobalt, copper, iron, manganese, molybdenum, selenium, and zinc are needed at low levels as catalysts for enzyme activities. The water containing high levels of these essential metals, or toxic metals such as aluminum, arsenic, barium, cadmium, chromium, lead, mercury, selenium, and silver, may behazardous to our health. Metals in our water supply may occur naturally or may be the result of contamination. Naturally occurring metals are dissolved in water when it comes into contact with rock or soil

Appendix of pictures to electron microscopic for samples from Al-Dalmaj marsh water in five stations



The low concentrations are probably related to the high pH values, which also seems to influence the concentration of these metals in natural unpolluted water (20).The prevalence of increased diseases were markedly increases in the last few

Other sources of metal contamination are corrosion of pipes and leakage from waste disposal sites. One of the major symptoms of chemical toxicity seems to be a breakdown of the immune system, which opens the gateway for all kinds of

diseases in the body. Also, another major symptom seems to be damage to the nervous system and increased nervousness. Toxic doses of chemicals cause either acute or chronic health effects. The levels of chemicals in drinking water, however, are seldom high enough to cause acute health effects. They are more likely to cause chronic health effects that occur long after exposure to small amounts .Pointed out that there was an inflow of water from the North into the Wash basin. Presumably, the predominant bacteria in the marsh will be derived from this mud and water.If large numbers of terrestrial bacteria are also swept into the area they must either be killed and replaced by marine forms or they must rapidly adapt themselves to saline conditions (15).

It is possible that in other estuaries where it is known that the mud is largely of terrestrial origin. Preliminary samples from Al-Dalmaj marsh water suggest that these contain rather more bacteria that would grow on media.

The data in Table 3 indicate that plate count and total numbers of bacteria are markedly elevated in marsh water suggest that the proportion of the surface population that is readily grown and, thus, metabolically active is greater than that in the subsurface. Although the plate count procedure is limited in the numbers and types of active bacteria detectable, it was quite useful for the purpose of assaying relative differences in surface layer populations.No attempt was made to determine all the metabolically active bacteria. When detection of such organisms is desired, other techniques, such as heterotrophic uptake. On the basis as the work described in this paper, an investigation into the distribution of bacteria in Al-Dalmaj marsh water .

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