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Estimation of selected heavy metals and bacteria in sludge of wastewater treatment unit at AL-Nassiryia city southern of Iraq

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Abstract:

The present study was concerned to estimation the concentrations of the selected heavy metals (Zinc, Copper, Lead, Nickel and Cadmium) in the sewage sludge, which was collecting from the wastewater treatment unit from the center of Al-Nassiriya city during the seasons of the year (autumn , winter , spring and summer) from October, 2016 to August, 2017. Three stations were chosen to execute this study, station (1): represent the pre-treatment; station (2): represent post-treatment and station (3) represent after dumping in the river. The results of the present study showed a high concentration of heavy metals in dry sludge was Zinc (91.86) µg/g dry wt. in the (st.1) in Spring season, while the Nickel (5.19) µg/g dry wt. at the (st.1) in Summer. The high concentration of copper (41.20) µg/g dry wt. at the (st.3) in Winter , and lead was (50.17) µg/g dry wt. at the (st.3) in Winter, and cadmium was (97.20) µg/g dry wt. at (st.2) in Winter. The high concentrations of fecal coliforms was (18000) cells /100 ml at (st.1) in winter, while the high number of total coliforms was (160000) cells/100 ml at (st.1) in winter. According to statistical analysis; The results of the present study showed a significant differences between the concentrations of heavy metals during the seasons of the year at the stations, as well as significant differences between the bacteria at the stations that were studied.

Keywords: Sludge, Heavy metals, Bacteria, Treatment unit.

Introduction:

Pollution is defined as an undesirable change in qualities, whether chemical, physical or biological, resulting in damage to human life and other organisms (Crompton, 1997). It is also defined as a set of physical, chemical and biological changes that change the quality of water and make it harmful to the environment and poses a threat to life and the environment (Ambedkar, 1999). Heavy metals are known as those with an atomic number of more than (20) and a density of more than (5) g / cm^3, also called trace elements because they are present in few concentrations in the earth's crust, not exceeding 0.1% (Tucker *et al*., 2003). Heavy metals are naturally found in the environment, widely used in manufacturing processes and are involved in the installation of the environment and are therefore transported to the remaining organic residues (Lineres, 1992). There are many sources of heavy metals in the sludge, especially products derived from household solid waste, these materials include household dust and batteries as well as disposable household items such as glass cutters, plastics, inks and paints (Bardos, 2004). Recently, body care products, medicines and household pesticides concerns about the contamination of the aquatic environment with heavy metals are increasing, the introduction of industrial waste containing heavy metals is a potential threat to the aquatic environment (Tang *et al* . , 2014). Effective removal of heavy metals from wastewater depends on several factors, concentration of sludge, melting of metal ions, pH, metals and their concentration , and the release of contaminants in sewage (Chipasa, 2003). The total content of heavy metals in sludge deposits usually varies within the limits (0.5 - 2.0%) of dry matter, the main source of heavy metals in sewage disposal is industrial wastewater, surface runoff and about (80 - 90%) of heavy metals flowing in the liquid waste collected from the sewage solution, heavy metals in sludge contents may vary depending on the source of the sewage (Babel *et al* ., 2006). The rivers in areas with different human activities, such as agricultural fields, cities and industrial plants, are often used as waste disposal sites, waste water, industrial waste and agricultural waste (Adewoye, 2010). Sewage is described as one of the most serious problems on public health in most third world countries, because most of these countries do not have an integrated sewage network, but use some channels to discharge, resulting in a large pollution and the spread of many diseases, as the sewage contains large amounts of organic compounds and a large number of aerobic and anaerobic microorganisms (Harold, 1998). Sewage is responsible for the transmission of various pathogens, such as bilharziasis, yellow fever, typhoid and others if water is used without integrated treatment (Harold, 1998). The calculation of the total number of coliforms is a sign of contamination of water with some fecal matter, where the presence of colonic bacteria in the water is evidence of the arrival of fecal matter and excretions in it, because these bacillus live naturally in the intestines of humans and animals (Thelin and Gifford, 2000). It includes four main genus (*Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella*), in addition to these species of this group included, some rare species that do not exist with feces with the possibility of breeding in drinking water of good quality, the most important of these types (*Serratia fonticolar*, *Robenella aquatitis and Battiauxella agrestis*) (WHO, 2000). Fecal coliforms constitute between (60-90%) of total coliforms, including (*Escherichia*, *Citrobacter* and *Klebsiella*). *Escherichia* constitutes (90%) of this group (Kress and Gifford, 2001). The aim of this study was to estimate the seasonal changes in some heavy metals and bacterial content of the sewage sludge.

Materials and methods:

1. Estimation of heavy metals in dry sludge:

Sludge samples were dried at 80°C for 48 hours after removal of solid parts and other dirty. A ceramic mortar was used and then passed through a sieve 63 μm mesh size, 1gm of the sample was took and placed in a tephlon baker and 6 ml of the concentrated hydrochloric and nitric acids mixture at a rate (1:1) were add then putting on a hot plate at 80°C until it was reached pre-dry phase, then added 4 ml of a mixture of hydrofluoric acid and perchloric acid at a rate (1:1) and placed on the hot plate 80°C until the pre-drought phase, then, samples were dissolved in 20 ml of hydrochloric acid 0.5 N. The samples were filtrated and putting in clear and clean plastic bottles 25 ml in size and volume was completed with de ionized distilled water and it was ready to be measured by using the flame atomic absorption spectrophotometer (Yi *et al*.,2007)

2. Total Coliform (TC):

Three groups of test tubes containing maCconkey broth were used. Each group consist of five tubes, the first group contained 10 ml of maCconkey broth media at a double concentration but the second and the third groups of tubes were filled with 10 ml from macconkey media in mono concentration and placed in each tubes above an inverted dirham tube to detected the formation of gas resulted from them. The three groups of tubes were inoculated with (0.1, 1.0 and 10) ml, respectively of samples and incubated at 37 °C for 24 hours and then, the positive tubes for each group were calculated as a result of the produce of gas and acid from positive growth. The negative tubes for each group were returned to the incubator and left for another 24 hours and the results were calculated according to special tables for MPN (APHA, 2003).

3. Fecal Coliform (FC):

The most probable number (MPN) method is a confirmed test of the positive reaction tubes in the previous test was carried out with three groups of maCconkey broth , tubes containing the monoconcentration by loop, the tubes were incubated at 44.5 °C for 24 hours, number of positive tubes (growth, gas and acid) according to the tables in the procedure at the source below, the most likely number of fecal coliforms (APHA, 2003).

4. Study area:

The study was conducted on the sewage treatment stations that were located at the center of Nassiriya city and it were lies at the left side of Euphrates river during the period from October, 2016 to August, 2017. The present study included three stations with three sites, before and after treatments, as well as when the waste dumping station in the river.

5. Statistical analysis:

The results were statistically analyzed according to the design of the factorial experiments, by two factors and three replications, the first factor was the study of stations (St.1, St.2 and St.3), the second factor was the seasons (autumn, winter, spring and summer) and using the statistical program SPSS-11-2003 (Statistical Package for Social Science) on the analysis of a results by using a least significant differences (L.S.D.) on the analysis of variance at probability level ($P \leq 0.05$).

Results:

1. Estimation of heavy metals concentrations in the dry sludge:

Table (1) shows the concentrations of heavy metals in the dry sludge during seasons , the high concentration of zinc was (91.86) µg / gm at the first station on spring and the low concentration was (29.72) µg / gm at the third station during summer season , the lead recorded the high concentration was (50.17) µg / gm at first station during winter and the low concentration was (0.67) µg / gm at the third station in summer, the high concentration of nickel was (5.19) µg / gm at first station in summer and the low concentration was (0.43) µg / gm at second station in summer, the high concentration of copper was (41.20) µg / gm at the third station in winter and the low concentration was (3.00) µg / gm at the third station in summer, cadmium had the high concentration (97.20) µg / gm at the second station in winter and the low concentration was (0.69) µg / gm at the third station in summer, from the general mean of heavy metals showing that, the concentration of zinc was the high (57.982) µg / gm, followed by cadmium

Table (1): Seasonal changes in concentrations (µg / gm dry weight)of heavy metals in dry sludge.

Cd	Cu	Ni	Pb	Zn	Stations	Season
f 70.80	g 22.70	cde 1.66	e 34.80	b 80.13	St.1	Autumn
b 91.80	h 18.60	defg 1.32	b 45.20	e 69.28	St.2	
d 81.60	f 24.67	hi 0.74	f 30.63	f 60.40	St.3	
d 81.40	g 21.99	defgh 1.24	cd 36.87	e 69.93	mean	
bc 89.45	c 33.48	fgh 1.02	a 50.17	bc 77.91	St.1	Winter
a 97.20	d 29.90	ghi 0.78	c 38.17	bc 79.70	St.2	
e 77.35	a 41.20	def 1.40	ij 19.75	d 74.65	St.3	
c 88.0	c 34.86	efgh 1.07	de 36.03	c 77.42	mean	
g 67.06	e 27.47	b 3.24	f 30.33	a 91.86	St.1	Spring
k 24.50	b 37.37	c 2.12	i 21.11	k 30.22	St.2	
l 21.22	i 14.06	i 0.22	j 19.20	k 30.26	St.3	
j 37.59	ef 26.30	cd 1.86	h 23.54	h 50.78	mean	
I 50.19	i 13.95	a 5.19	k 15.56	i 40.26	St.1	Summer
n 0.71	k 3.69	i 0.43	l 10.0	k 31.42	St.2	
n 0.69	k 3.0	i 0.44	m 0.67	k 29.72	St.3	
m 17.28	j 6.88	c 2.02	l 8.74	j 33.80	mean	
56.067	22.507	1.547	26.295	57.982	General mean	
2.87	1.69	0.57	1.84	2.35	L.S.D	

(56.067) µg / gm, lead (26.295) µg / gm, copper (22.507) µg / gm and nickel (1.547) µg / gm, also the concentrations of metals that were mentioned, its a high at the first and second stations, whereas the low observed on the third station for all seasons of the year. There were significant differences observed between concentrations of metals at stations and between seasons.

2. Numbers of fecal coliforms:

Table (2) were showing numbers of fecal coliforms and illustrates the seasonal changes in their samples of dry sludge samples. In the first station, the high rate of bacilli number was (18000) cells / 100 ml during winter season, while the low rate number was (3400) during autumn season, In the second station, the high number of fecal coliforms was (11000) cells / 100 ml recorded during winter season, while the low rate number was (900) cells / 100 ml observed during summer, in the third station, the high rate number of fecal coliforms was

Table (2): total number of fecal coliforms (cells / 100 ml) in dry sludge during four seasons.

Summer	Spring	Winter	Autumn	Seasons	
g 8000	b 17000	a 18000	j 3400	St.1	Station
p 900	h 4900	e 11000	m 2700	St.2	
q 700	k 3300	g 8000	o 2300	St.3	
l 3200	f 8400	c 12333.33	m 2800	mean	
37.16				L.S.D	

(8000) cells / 100 ml during winter and the low rate number was (700) cells / 100 ml during the summer. From a results of the statistical analysis, it were observed significant differences between the total number of fecal coliform bacteria at the stations on the all seasons.

3. Number of total coliforms:

Table (3) shows the number of total coliforms and the seasonal changes in their numbers for dry sludge samples, at the first station, the high rate of bacilli was (160000) cells / 100 ml during winter season, while the low rate was (28000) cells / 100 ml observed during summer, at the second station, the high number of coliforms was recorded (92000) cell / 100 ml in winter, while the low rate was (9400) cells / 100 ml during summer , and the third station recorded the high rate of coliforms was (24000) cells / 100 ml during winter and the low rate (4600) cells / 100 ml during summer. The results of present study were showing differences between numbers of coliforms at stations and seasons and significant differences were noticed.

Table (3):Total number of total coliforms (cells / 100 ml) in dry sludge during the four seasons .

Summer	Spring	Winter	Autumn	Seasons	
c 28000	*d* 54000	*a* 160000	*f* 35000	St.1	Station
o 9400	*h* 28000	*b* 92000	*j* 24000	St.2	
p 4600	*h* 13000	*j* 24000	*k* 22000	St.3	
m 14000	*g* 31666.66	*b* 92000	*i* 27000	mean	
101.32				L.S.D	

Discussion:

1. Heavy metals in dry sludge:

The concentrations of heavy metals in a dry sludge for Zinc, Lead, Nickel, Copper and Cadmium were (57.985, 26.295, 1.547, 22.507, and 56.067) µg / gm of dry weight respectively, the increase on these concentrations of heavy metals may be attributed to the fall of pollutants from the atmosphere with dust particles which due to the dust storms in the region during the year, these results are in agreement with (Almuddafr *et al* . , 1992), who refers in their study to the distribution of heavy metals in the Shatt al-Arab, Basrah deposits, and attributed the reason to the dust storms that are frequent in the region and lead to the fall of pollutants from the atmosphere. The discharge of sewage, industrial waste and drifts from cities, as well as atmospheric deposition, is one of the most important sources of heavy metals contaminated with sediments (Kumar *et al* ., 2011). When the results in the current study were compared with the results of others such as (Hinesly and Hansen, 1983 ; Hinesly *et al* . , 1984) showing that, its corresponding to that were recorded values, with the exception of cadmium and this result may be due to the fact that, the sewage is loaded with phosphate fertilizers, which was an important source of cadmium (Kabata, 1989). The results were showing also, the increases in heavy metals concentration at all the studied stations during winter, this may be due to the large quantities of heavy metals in the waste water, or from the agricultural areas or untreated industrial wastewater, in particular the electric power station's implications and the obvious effect on the increases of heavy metal concentrations, In addition, the sediments formed from the erosion of the edges of the rivers or high areas from which the rain descends to the nearby stream of its location (Brayner *et al* . , 2003).

Many environmental and anthropogenic factors plays an important roles in the deposition of heavy metals and plankton from the water column to the bottom, these factors were including salinity, amount of organic matter in sediments, surface area of adsorption and current velocity (Salvado *et al* . , 2006). When comparing the concentration (µg/g dry weight) of heavy metal of sediments to the current study with some of the standard specifications such as (EPA, 2004), the concentrations of metals studied (Copper, Zinc, Nickel and Lead) are not polluted, whereas Cadmium was highly polluted.

2. Number of fecal coliforms:

The detection of fecal coliforms was the best guide to determination the contamination of sewage waste with fecal material, and it was presence indicates the possibility of pathogens in the water (Edbertg *et al* . , 2000). The results were showing a clear variations on numbers of fecal coliforms bacteria of the studied stations. The number of fecal coliforms at the first and second stations were high and low it was at the third station which noticed during the seasons, this may be attributed to the dumping of untreated wastewater (no treatment of bacteria in wastewater treatment stations) that contain large numbers of bacteria, while the third station had a high density of plants that acts as filters and the process of dilution of pollutants on the river, and it was noticed from the results that, the high numbers of these germs in winter and low in summer may be attributed to decline during summer because of high temperature and water impact of the sun that killing the large numbers, while the low temperature works to keep these germs for a period

(Atlas *et al* . , 1995). The discharge of sewage water on the river works to reduces microorganisms (Gbaruko and Friday, 2007), these results are accordance with (Martha *et al* . , 1985) finding in their study of tropical rainforests coasts. The results of the study were showing that, the discharge of sewage had led to an increase in the number of bacteria pollution, as numbers of these bacteria exceeded the Iraqi and global determinants of water used for drinking.

3.Number of total coliforms:

The results showed that, the number of total coliforms at the first and second stations increased in compared with the third station during seasons of the year, it may be due to the dumping of untreated sewage waste (no treatment of bacteria in sewage treatment station) which contain large numbers of bacteria, while the third station had a high plant density that works water filtration as well as the process of reducing the waste by the dilution in the river (Gbaruko and Friday, 2007). The results were showing that, the high numbers noticed on winter and the low during summer, the low number of coliforms during summer may be attributed to high temperature of water and the effect of the sun light and the length of day, which causes a death to large numbers bacteria (Ria and Hill, 1978), whereas low temperature was working to conserve the bacteria as longer (Atlas *et al* . , 1995). These results are in accordance with (Martha *et al* . , 1985) in its study of tropical rainforests coasts.

Conclusion:

According the present study results, the study may conclude that the dry sludge in AL-Nassiriya City had a large content of heavy metal and bacteria as compared with (US-EPA, 2004).

Table (4): Levels of heavy metal contamination (mg / kg) in sediments according to US environmental protection standards.

Heavy metals	Unpolluted	Medium polluted	Highly polluted
Cadmium	-	-	6 <
Copper	25 >	50-25	50 <
Nickel	20 >	50-20	50 <
Lead	40 >	60-40	60 <

Table (5): Comparison of heavy metals concentrations (µg / g) dry weight of dry sludge with some standard parameters

Heavy metals	(NOAA, 2000)	(CSQG, 2002)	Present study
Cadmium	110.00 - 9.60	3.50 - 0.60	56.067
Copper	-	-	22.507
Nickel	51.60 - 20.90	-	1.547
Lead	218.00 - 46.70	91.30 - 35.00	26.295
Zinc	410.00 - 150.00	315.00 - 123.00	57.982

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