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Study Capacity of Reeds and Papyrus Plants in Bioremediation for Removing Some Excreted Antibiotics to Waste Water for Samarra Drug Industry

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ABSTRACT

This research included study the capacity of *Typha domengensis* and *Phragmites communis* plants in bioremediation for removing some antibiotics as one of pollutants discard to Waste Water for Samarra Drug Industry (SDI) two antibiotics had selected (Cephalexin and Amoxicillin) because their amounts is the greater according to production during study period. Two stations had Selected for test, The first station of exist plants inside Samarra drug industry and the second station of plants exist on the edge of the Tigris river as control station. The study included the evaluation of antibiotics, chlorophyll A and B, proteins and carbohydrates in plants.

The results of the plants indicated that the concentrations of antibiotics in the first station ranged between (0.002-0.089) mg / ml for cephalexin and (Nil-0.001) mg / ml for amoxicillin in reeds, and results ranged between (0.001-0.095) mg / ml for cephalexin and was not significant (Nil) for amoxicillin in papyrus, and no antibiotic value (Nil) was recorded at the second station. The values of chlorophyll A ranged (0.18-1.09), (0.12-0.89) mg / L at the first and second stations respectively, as well as the values of chlorophyll B ranged between (0.36-2.38), (0.28-1.46) mg / L for reeds, There was also variability in papyrus, with values of chlorophyll a (0.05-0.99), (0.17-1.92) mg / L and chlorophyll B ranged between (0.20-1.67), (0.40-1.76) mg / L for both stations respectively, The values of the proteins in the reeds ranged between (250-783), (184-680) µg/L respectively, and in the papyrus were (330-673), (280-726) µg/L for the two stations respectively, Carbohydrate values in reeds ranged between (0.16-2.60), (0.11-2.57) mg/L respectively, and in the papyrus was between (0.15-2.88), (0.13-2.59) mg/L respectively.

دراسة قدرة نباتي القصب *Phragmites communis* والبردي *Typha domengensis* في المعالجة الحيوية لإزالة بعض المضادات الحيوية المطروحة في مياه الصرف لمعمل ادوية سامراء.

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الخلاصة

تضمن البحث دراسة القدرة الحيوية لنباتي القصب والبردي للتخلص من بعض المضادات الحيوية كأحد الملوثات المطروحة مع مياه فضلات معمل ادوية سامراء، تم اختيار نوعين من المضادات الحيوية السيفالكسين Cephalexin والاموكسيسلين Amoxicillin كونها الاكثر من حيث الانتاج خلال فترة البحث. تم اختيار محطتين للقياس الاولى من النباتات المتواجدة داخل معمل ادوية سامراء والمحطة الثانية من النباتات المتواجدة على حافة نهر دجلة كمحطة سيطرة. شملت الدراسة قياس المضادات الحيوية، الكلوروفيل أ ، ب ، البروتينات، الكاربوهيدرات في النباتات. واشارت النتائج الى ان تراكيز المضادات الحيوية في نباتات المحطة الاولى تراوحت بين (0.002–0.089) ملغم/مل للسيفالكسين و(Nil -0.001) ملغم/مل للاموكسيسلين في القصب، وتراوحت بين (0.001–0.095) ملغم/مل للسيفالكسين وغير محسوسة (ND) للاموكسيسلين في البردي، لم تسجل اي قيمة للمضادات الحيوية (ND) في المحطة

[1] This paper is quoted from Ph. D. thesis for the first searcher.

الثانية. كما وتباينت قيم كلوروفيل أ بين (0.18–1.09)،(0.12–0.89) ملغم/لتر في المحطة الاولى والثانية على التتابع، وكذلك قيم كلوروفيل ب بين (0.36–2.38)،(0.28–1.46) ملغم/لتر لنبات القصب، كما وسجلت كذلك تباينا في البردي اذ تراوحت قيم كلوروفيل أ بين (0.05–0.99)،(0.17–1.92) ملغم/لتر ، والكلوروفيل ب تراوحت بين (0.20–1.67)،(0.40–1.76) ملغم/لتر للمحطتين على التتابع، كما وتباينت قيم البروتينات في القصب بين (250–783)،(184–680) مايكرو غرام/لتر ، وفي البردي كانت بين (330–673)،(280–726) مايكرو غرام/لتر للمحطتين على التتابع، وكذلك قيم الكاربوهيدرات في القصب بين (0.16–2.60)، (0.11–2.57) ملغم/لتر ، وفي البردي كانت (0.15–2.88)،(0.13–2.59) ملغم/لتر للمحطتين على التتابع.

Introduction:

Bioremediation is defined as specialized treatment to remove contaminants from domestic or industrial waste water and convert it into less polluted and safer water, or treat it so as not to pose a threat to humans and the environment (Alsheikhle, 2000). Bioremediation is one of the most effective and modern methods of low-cost, bio-technology, which aims to use different organisms in the removal of contaminants and control of pollution. The presence of the organism is the fundamental and important element in the biological process (Nasrati, *et al*., 2006).

In addition to complex biological, physical and other chemical processes that are carried out in microbiological treatment basins or both, in order to improve wastewater characteristics, these mechanisms depend on the reciprocal interactions between sewage and microorganisms on the one hand and plants (Baban, *et al*., 2004).The Papyrus and Reeds are commonly used in plant treatment plants. The plants that grow within these environments have many advantages for the treatment process (Al-Hadithi, *et al*., 2016).

Plants play a number of roles in treatment basins, but their main role is the nature of their work as a tool or means of purification. The purification process results from a combination of microbial, chemical and physical processes (Swearingen and Saltonstall, 2010), and plants do not play an important role in the direct removal of certain components such as nitrogen and phosphorus or organic matter but contribute to the elimination of 10-20% of them during the growth period and at the same time, they provide significant support for bacterial growth in the root zone (Mitsch, 2004).

Amoxicillin an antibiotic of the family of penicillin is widely used and broad spectrum and is toxic in a simple manner in the environment studies indicate that some factors such as direct light can be responsible for the transformation and removal of amoxicillin from the aquatic environment, and biodegradation and active mud is a good way to remove it (Andreozzi, *et al*., 2003). The accumulation of small quantities of antibiotics may cause real problems in the long term, so they should be investigated continuously and knowledge of the behavior of these substances under normal conditions and the extent of their toxicity to living organisms, and this is essential in predicting environmental hazard (Leung, 2001).

Materials and methods of work:

The concentration of chlorophyll dye (A, B) was measured for the studied plant samples according to method (Mackinny, 1941), and the concentration of chlorophyll units Mg / g) was measured according to the following equations:

Concentration of chlorophyll A = (12.7 x 663 absorbance) - (2.69 x 645 absorption) /15 ml.

$$\textbf{Chlorophyll Concentration b} = \frac{V * (663a * 4.68) - (645a - 22.9)}{W * 1000}$$

Where: V = volume of solvent (Aston). W = the weight of the sample.

We measure the sugars in the plants according to method (Dubois, *et al*., 1956), glucose absorbance is measuring and dropping the absorbance values of the samples on diagrams to measure and deduce concentrations of sugars of plant samples.

The protein values of the plants measures according to method (Lowry, *et al*., 1951).

We use the optical spectrometer to measure absorption at a wavelength of 750 nm. The standard solution dissolves 5 milligrams of protein in 25 ml of distilled water, and then take certain dilute concentrations with distilled water for the standard curve drawing. The concentration of the protein determines according to the following law: (1) II and then draw the standard curve and drop the absorption of the samples on the measured concentrations.

The antimicrobial concentrations of Cephalexin and Amoxicillin calculate for US plant-treated samples (USP-NF 38, 2015) and HPLC (Perkin-Perkin-US). Antibiotics concentrations in plants measure by examining the plant sample immediately after taking it from the study site and transferring it to the laboratory and prepared to be injected directly into the HPLC device, and the ratio of antibiotics measures by the emergence of Peak of each type compared to the standard concentration measured at 0.1 mg / ml. And calculates according to the following law:

$$\frac{\textbf{Concentration of standard}}{\textbf{Peak area of the standard}} = \frac{\textbf{Sample Concentration}}{\textbf{Peak area of sample}}$$

Results and discussion:

The current study shows that chlorophyll A concentrations are lower than the levels of chlorophyll B concentrations for reeds and papyrus plants and for both stations (Table 1). The highest value of chlorophyll A reed plant in the first station is 1.09 mg/g during July 2016, The lowest value of chlorophyll A is 0.18 mg/g during the month of March 2016, while the highest value in the second station (0.89) mg/g in December of 2015, and come a value of 0.12 mg/g March 2016. The highest value of chlorophyll A papyrus in the first station amounted to (1.99) mg/g during the month of November of 2015, and recorded The lowest value of chlorophyll A was (0.05) mg/g during the month of March 2016, while the highest value in the second station (1.92) mg/g in the month of November of 2015, and reached a value of 0.17 mg/g Of 2016.

The lowest values for chlorophyll A for reed and berry plants for all stations in the same month are shown in the present study. The highest values of chlorophyll A were also agreed in papyrus for the first and second plants, but the highest values of chlorophyll A in reed plants varied in different months. The statistical analysis showed significant differences of chlorophyll between the two plants at a significant level by Person Variance Analysis ($p \leq 0.05$).

The results of the present study found that the concentration of chlorophyll A for reed plant in the first plant contaminated with wastewater from the industrial wastewater of Samarra compared to the second station of the Tigris River is increasing, according to the quantity, quality and concentration of these reagents (Hadad, *et al*., 2010). The concentration of chlorophyll is under the influence of the increase or decrease in concentration of pollutants (Mary, 2008). The low concentration of chlorophyll is due to the negative effect of metals on the absorption of magnesium, which enters the structure of chlorophyll (Shukla, *et al*., 2010). The effect of metal contaminants inhibits the bio-construction of chlorophyll prior to the protochlorophyllide phase because of its overlap with the enzyme Reductase Protochlorophyllide (Zengin and Munzuroglu, 2005).

The decrease in chlorophyll A concentration is also due to an internal substitution of the magnesium atom in the center of the chlorophyll molecule with the heavy metal seed, which is an important destruction mechanism in the plants (Kasim, 2005). Therefore, the reduction of chlorophyll content is due to the low concentration of magnesium absorbed by the plants. The study agreed with the findings (Al-Janabi, 2000), which indicated that soil contaminated with oil residues reduced the amount of chlorophyll in plants and impaired photosynthesis.

The highest value of chlorophyll for reed plant was at 2.38 mg / g during the month of July 2016. The lowest value of chlorophyll was 0.36 mg / g during the month of January 2016. The highest value was in the second station (0.48) mg / g during the month of January 2016. The highest value of chlorophyll for papyrus plant in the first station amounted to (1.67) mg / g during the month of July of (0.20) mg / g during the month of March 2016, while the highest value in the second plant was (1.76) mg / g in the month of November For the year 2015, and reached a value of (0.40) mg / g during the month of March of 2016.

The results of the current study show that chlorophyll B is less sensitive than chlorophyll A by its contaminants because of the fluctuation in its results. It is likely that its increase in contaminated areas indicates the susceptibility of plants to resist pollution by increasing photosynthesis and increasing nutrient uptake , And the protein ratios are high indicating the plant's viability and endurance, as well as the high concentrations of summer in the temperature of the effect of increased breathing, construction and absorption.

This is consistent with the study of (Vassilev and Yordanov, 1997) which indicated an increase in the sensitivity of chlorophyll A compared with chlorophyll B, and the reduction of chlorophyll B is due to the presence of industrial waste, which leads to changes in the number of karana and content of starch granules and the fatty compounds involved in the formation of coranas Suffer from diminution or decomposition or rupture of the membrane surrounding the chloroplast (Das, *et al*., 1998) .

(Table – 1): Contains of chlorophyll for reeds and Papyrus plants in tow station

Type of plant		Chlorophyll A				Chlorophyll B			
		Reeds		Papyrus		Reeds		Papyrus	
Stations Months		First Station	Second Station	First Station	Second Station	First Station	Second Station	First Station	Second Station
2015	9	0.58	0.26	0.61	0.63	0.90	0.40	1.03	0.72
	10	0.66	0.55	0.80	0.92	0.97	0.74	1.29	1.24
	11	0.73	0.84	0.99	1.92	1.79	1.07	1.54	1.76
	12	0.61	0.89	0.87	0.62	0.67	0.93	0.97	0.50
2016	1	0.28	0.23	0.52	0.73	0.36	0.28	0.71	0.76
	2	0.23	0.18	0.28	0.45	0.39	0.30	0.57	0.72
	3	0.18	0.12	0.05	0.17	0.42	0.33	0.20	0.40
	4	0.28	0.35	0.27	0.30	0.51	0.58	0.47	0.56
	5	0.39	0.58	0.50	0.43	0.60	0.84	0.74	0.71
	6	0.81	0.66	0.60	0.54	1.49	1.15	1.21	0.97
	7	1.09	0.76	0.80	0.66	2.38	1.46	1.67	1.23
	8	0.93	0.68	0.77	0.52	1.93	1.31	1.44	1.10
Mean		0.56	0.50	0.58	0.65	1.03	0.78	0.99	0.89

The highest value of carbohydrates for reed plant in the first station are 2.60 mg/ml in May 2016. The lowest value of carbohydrates was (0.16) mg/ml during the month of November of 2015, while the highest value in the second station (2.57) In the month of March 2016, and the lowest value is (0.11) mg/ml during the month of November of 2015. The highest value of carbohydrates for papyrus plant in the first station amounted to (2.88) mg/ml in June of 2016, and recorded the lowest value of carbohydrates (0.15) mg/ml during the month of November 2015, while the highest value in the second station (2.59) in January For the year 2016, with a value of 0.13 mg/ml during the month of November 2015 (Table 2).

The current study shows that the minimum carbohydrate values of reeds and papyrus plants were identical for all stations in the same month. The highest values in reeds and papyrus did not correspond to the first and second plants. The statistical analysis shows significant differences of carbohydrates between the two plants at a significant level ($p \leq 0.05$).

Plants planted in soils exposed to industrial waste are subject to a decrease in the concentration of carbohydrates. This is due to the negative effect of pollutants in the process of photosynthesis and the transfer and production of carbohydrates in the tissues of plants (Kumar and Sadhna, 1999), and thus the effect of regulating the process of opening and closing the gaps. The lack of plant vegetation leads to a reduction in the amount of carbohydrates produced by photosynthesis (Yruela, 2009).

The present study did not agree with what was reached (Arabi, 2017). The increase in the amount of chlorophyll affect the process of photosynthesis, thus increasing the composition of carbohydrates, in addition to increasing the components of the plant of sugars contributes to the acquisition of a large vegetative and root, in addition to the release of ions (hydrogen ion) to the soil, Which facilitates nutrient uptake by plants (Erdini, 2016). The current study did not agree with the findings of the present study (Mohammed, 2007). The higher values of the current study were much lower than in the study of the effect of soils contaminated with oil residues on the growth and productivity of two varieties of wheat and barley.

The highest value of the reed plant protein was recorded in the first plant (783) μg/ml during the month of January 2016. The lowest value of the proteins was 250 μg/ml during September of 2015. The highest value of reeds at the second station (680) In the month of March 2016, with a value of 184 micrograms/ml during the month of November 2015. The highest value of papyrus proteins in the first station was 673 micrograms/ml in December of 2015 (330 μg/ml) during the month of November of 2015, while the highest value was in water Plan II (726) mcg/ml in July 2016, and was the lowest value (280) mcg/ml for the month of November for the year 2015 (Table 2). The current study shows that the minimum values of the reed plant proteins in the second plant and the papyrus plant in the two plants of the same month were consistent with the highest values in the reeds and papyrus plants of the first and second plants.

The effect of stress, whether water or salt or low temperature, is based on the formation of Abyssic, which controls the growth and development of the plant, as well as controls many physiological processes such as closing the gaps and formation of the fetus and composition of seeds and composition of protein stocks and fat and germination and aging and defend the cell against microbes and parasites also plays Ephesics plays an important role in plant adaptation to environmental conditions, and plants exposed to high temperatures cause severe changes in food transitions and the formation of new types of protein, known as heat shock proteins (Saqr, 2011).

If the plants are exposed to temperatures above the optimal limit by about 5 m, the cells are also destroyed and the plasma membranes are damaged (Dipti *et al*., 2016) indicated that the rate of protein decreases in plants growing in industrial areas by 5-20%. This decrease is explained by the degradation of the protein found in plant tissues. The present study agreed with (Arabi,2017).

Antibiotics were measured in the current study. It was found that the highest value of cephalexin at the first plant was 0.089 mg/ml during December of 2015 and the lowest value of cephalexin was 0.002 mg/ml in the first station during the month of January 2016 , While there was no significant value of amoxicillin and cephalexin in most periods of study of reeds and papyrus plants and both plants, and recorded only one value (0.001) mg/ml during the month of March in the first station of 2016, and this indicates that the concentration of amoxicillin was not significant in most Periods of study, and it also indicates access to amoxicillin To the plant through the former treatment plants, as is the case with cephalexin (Table 3), (Diagram 1).

(Table – 2): The value of carbohydrates and proteins of reed and papyrus

Type of plant		Carbohydrate				Proteins			
		Reeds		Papyrus		Reeds		Papyrus	
Stations Months		First Station	Second Station	First Station	Second Station	First Station	Second Station	First Station	Second Station
2015	9	0.67	0.47	0.56	0.14	250	530	645	521
	10	0.41	0.29	0.36	0.13	252	327	522	453
	11	0.16	0.11	0.15	0.13	254	184	330	280
	12	0.52	0.44	0.49	0.45	520	626	673	485
2016	1	2.40	2.40	2.58	2.59	783	630	488	252
	2	2.06	2.48	2.00	2.57	762	650	510	560
	3	1.73	2.57	1.42	2.56	574	680	540	590
	4	2.16	2.56	2.02	2.57	542	520	626	546
	5	2.60	2.55	2.63	2.58	504	326	645	470
	6	2.24	2.50	2.88	2.50	508	521	620	623
	7	1.89	2.47	2.35	2.49	524	650	525	726
	8	1.77	2.45	2.51	1.44	520	622	540	647
Mean		1.55	1.77	1.66	1.76	461.5	522.1	555.3	535.50

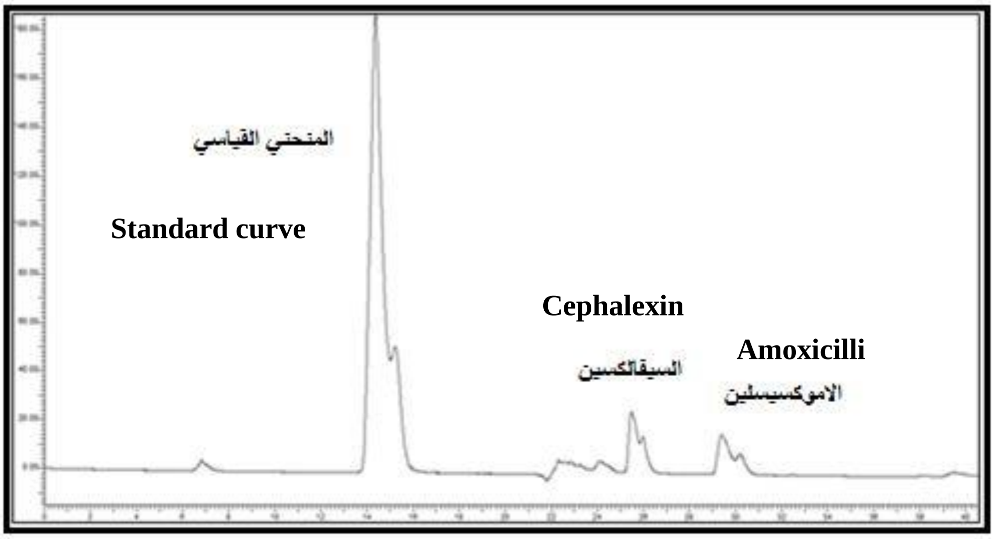

Diagram (1) A model of the studied curves for the measurement of antibiotics (Cephalexin and Amoxicillin).

(Table – 3): Some plant tests The value of Antibiotics Cephalexin and Amoxicillin

Type of plant		Cephalexin				Amoxicillin			
		Reeds		Papyrus		Reeds		Papyrus	
Months \ Stations		First Station	Second Station	First Station	Second Station	First Station	Second Station	First Station	Second Station
2015	9	0.040	Nil	0.030	Nil	Nil	Nil	Nil	Nil
	10	0.026	Nil	0.015	Nil	Nil	Nil	Nil	Nil
	11	0.009	Nil	0.007	Nil	Nil	Nil	Nil	Nil
	12	0.089	Nil	0.095	Nil	Nil	Nil	Nil	Nil
2016	1	0.002	Nil	0.001	Nil	Nil	Nil	Nil	Nil
	2	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	3	Nil	Nil	Nil	Nil	0.001	Nil	Nil	Nil
	4	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	5	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	6	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	7	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	8	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mean		0.0326	Nil	0.0296	Nil	0.001	Nil	Nil	Nil

The highest value of cephalexin in papyrus was at 0.095 mg/ml during December 2015, with the lowest value of cephalexin (0.001 mg/ml) in the first station in January 2016, No concentration of amoxicillin and cephalexin was recorded, meaning that their values were not discernible in the second station, which represents the control station. The present study agreed with (Christian, *et al*., 2003), in his study on the fate of antibiotics in soils and surface waters.

The study show that the highest and lowest values of antibiotic for both reeds and papyrus plants were observed for both plants in the same month and for the same year. The statistical analysis indicated significant differences of cephalexin between the two plants, while amoxicillin showed no significant differences at $p \leq 0.05$. The present study did not agree with the findings of (Alwan, 2016) studies of the efficiency of reeds and papyrus in removing pollutants from fresh water deposits.

The release of industrial pollutants containing antibiotics such as amoxicillin and cephalexin from non-point sources and mixing with contaminants and human waste has been a major problem in the treatment process, especially in aquatic environments (Yang and Carlson, 2003), so it cannot be removed by traditional methods of treatment (Heberer, 2002). Removal of pharmaceutical contaminants (Nghiem, *et al*., 2005). This type of plant treatment was used to remove most of the antibiotics (amoxicillin and cephalexin) used in Iran, which was referred to (Mehrania, *et al*., 2016) in his study on the qualitative evaluation of antibiotics in plant biological treatment plants. The percentage of removal of amoxicillin from wastewater was 86.5-88 And that of cephalexin was 74-88%. This is in line with the present study, which may explain the variation in antibiotic results, where cephalexin concentrations were significantly higher than those of amoxicillin concentrations.

The waste water contains a lot of impurities and suspended materials that may form a solid layer on the surface of the soil inhibits the arrival of antibiotics to the roots and thus lack of access to the plant, and this may explain the low values of antibiotics in the plants studied, and most of the values recorded in the chapter Winter, which indicates a decrease in their biodegradability (Stepanauskas, *et al*., 2006), or degradation due to the hardening of the surface of the soil due to the large temperature drop, or as a result of the lack of antibiotic-containing coatings (Qi Tang, et al., 2007). Antioxidants are directly exposed to sunlight and have viscosity when dissolved in water(Lee, *et al*., 2006). This advantage is exploited by algae and some bacteria and fungus as an adjunct to adhesion

(Lee and Elimelech, 2006). This adhesion is strong enough to maintain the structure of the organism to be protective gel membrane. Layered walls with basin walls (Zhou, *et al.*, 2006).

The increase in the proportion of antibiotics registered in the current study is due to the increase in the quantity of offerings, which comes from washing machines, equipment and floors, as well as the Samarra pharmaceutical factory was producing Cephalexin products over the months that showed concentrations in the plant, which helped to accumulate material In the long run, while the production of amoxicillin at varying intervals and short, and Amoxicillin more in the ability to water solubility of Cephalexin, all due to factors including temperature, pH, as well as the speed of waste water raised, Bactericidal agents are able to decompose a large part of antibiotics, while antibiotics act on the possibility of genetic imbalance and the emergence of antibiotic-resistant bacteria (Baquero, *et al*., 2008). The current study agreed with a study (Hamad and Dates, 2012) on evaluating the performance of the waste treatment plant in Nineveh. The appearance of bacteria resistant to antibiotics is considered to be a pollutant of the environment either in water or soil or affecting human and animal health, causing diseases different from those treated with the usual antibiotics (Koike, *et al*., 2007). This is consistent with the study of (Abdel Mawla, 2016) pollutants from the hospital Yarmouk.

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