

# Cytotoxic Effects of Al-Yohedia Municipal Waste Water

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## Abstract

Household waste water represent an hazardous reservoir for wide range of medically important pathogens than can push an alarm on public health threatening diseases. It contain toxic materials, hazardous heavy metals and different pathogens. The study includes investigation of microbial, environmental, pathological and molecular effects of waste water. The results of dissolved heavy metals concentration revealed high Ni concentration (Mean±SD of four sites were 119.675±0.778 mg/L), high Mn concentration (Mean±SD of four sites were 108.2±1.095 mg/L), high Fe concentration (Mean±SD of four sites were 119.675±0.740.475±1.228 mg/L), high Cd concentration (Mean±SD of four sites were 13.75±1mg/L), high Cu concentration (Mean±SD of four sites were 5±0.755mg/L) with no significant differences in concentration among four sites for Ni and Cu while difference noted among Mn, Fe and Cd. Results of microbial study revealed that the Mean±SD of CFU/ml were 1.97×10<sup>10</sup>±0.543 and the microbes were *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *V. cholerae*, *V. parahaemolyticus*, *S. aureus*, *E. faecalis*, *C. albicans*, *C. krusei* and *C. glabrata* with MAR index ranged from (0-1). DNA damage results using comet assay for revealed that high level of damage to DNA of rat dosed with waste water compared with those dosed with fresh water in both tail length (11.35±2.1, 2.7±0.9) and tail moment (1.193±0.11, 0.2±0.1) respectively. Immunohistochemistry (IHC) assay was used to detect of 8-OHDG among liver tissues of rat dosed with waste water (study) and fresh water (control) and the results revealed strong signal (high expression) in all liver tissues of rat dosed with waste water (study) compared with those dosed with fresh water (control) whose gave negative results. Histopathological results of eat dosed with waste water compared with those dosed with fresh water (control) were observed after 60 days of dosing. the results displayed that the control was normal where mild chronic hepatitis showed in samples of site 1, 2, 3 while site4 had moderate chronic hepatitis and *Fasciola hepatica*. The current study conclude that, Al-Yohedia Municipal wastewater is very dangerous and have different clinically important pathogens along with different cytotoxic materials.

**Keywords:** wastewater, *Vibrio cholerae*, *Candida* spp., Hepatitis, *Fasciola hepatica*

## Introduction

Municipal wastewater is a collection of both domestic and industrial waste water. Al-Yohedia is fresh water stream pass through Hilla city but unfortunately it serve as municipal waste water reservoir. Sewage water, healthcare clinics, butchers waste, diesel engine waste and farm animal waste were delivered directly to this stream rendering it dangerous spot of pollution [1,2]. The compartments of wastewater includes biological and non-biological parts. The biological part includes

bacteria, fungus, viruses and parasites while non-biological includes chemicals (organic and inorganic compounds like hydrocarbons and heavy metals) which pose dangerous impact on aquatic environments and human health [3-8]. Enterococci and enterobacteriaceae members like *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella* spp., *Shigella* spp., *Enterobacter* spp. were common among waste water while *Pseudomonas* spp., and *Vibrio* spp. push an alert of clinically important bacteria that cause life threaten diseases [9-13]. The presence of medically important yeast like *Candida* spp. among waste water were also stated. Human and animal waste water can be loaded with viruses like hepatitis and parasite like *Fasciola*

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hepatica and this proposing biohazard when reach to human being [6,14].

Cytotoxicity of waste water can be reflected and oxidative damage to biomolecules like to lipids of cellular membranes, proteins, and DNA which may lead to carcinogenesis. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is one of the predominant forms of free radical-induced oxidative lesions, and has therefore been widely used as a biomarker for oxidative stress and carcinogenesis. 8-OHdG used to estimate the DNA damage in humans after exposure to cancer-causing agents, such as tobacco smoke, asbestos fibers, heavy metals, and polycyclic aromatic hydrocarbons. In recent years, 8-OHdG has been used widely in many studies not only as a biomarker for the measurement of endogenous oxidative DNA damage but also as a risk factor for many diseases including cancer. Oxidative DNA damage is common in various forms of chronic liver disease suggesting a possible link between chronic inflammation and hepatocarcinogenesis. Heavy metals and some metalloids are known to be carcinogenic to humans. The oxidative concept in metal carcinogens were involvement of redox mechanisms production of hydroxyl radicals can attack DNA causing hydroxylation and oxidative DNA damage these products of DNA damage (including 8-OHdG) induce mutations leading to neoplastic transformations [15-18]. The current study aimed to investigate the cytotoxic effects of Al-Yohedia municipal waste water by IHC of 8-OHdG and histopathology of Rat lab animals.

## Materials and Methods

### Sampling:

Four waste water samples were collected from Al-Yohedia Stream. The study area included 4 different sites from Al-Yohedia stream in Hilla city (site 1 Al-karama quarter (N 32 0 28.944 E044 0 25.057), Site 2 Al-Jameia quarter (N32 0 28.383 E044 025.067), Site 3 Al-Iskan quarter (N32 0 27.545 E044 025.058), Site 4 Al-Askari quarter (N32 0 25.827 E044 024.394).

### Waste water Culture:

All samples mixed well and 1 ml of waste water sample transferred to 9 ml of normal saline (diluent) (Stock 10-1) and from it prepare five serial dilutions

(10-2, 10-3, 10-4 and 10-5) were prepared. 10 µl from the last dilution (fifth dilution) were spreaded on nutrient agar plate by disposable spreader and the plates were incubated upside down in 37°C for 24 hr. Triplicates were performed to each sample to increase the accuracy and only plate that has (30-300) colonies will be chosen for count. The exact number of colonies per original sample will calculated according to the following equation: CFU/ml = No. of colonies x Inverse of the dilution x 100<sup>[19]</sup>.

### Microbial Isolations and Identification:

Immediately after sample collection and along with total plate count the samples inoculated UTI chromogenic agar, Pseudomonas chromogenic agar, m-EI chromogenic agar Vibrio chromogenic agar and Candida chromogenic agar to investigate the presence of common pathogens among wastewater samples. The sample mixed well and from each sample a triplicate of 100 ml will filtrated on (0.45 µm) cellulose nitrate membranes (Sartorius/Germany) using Sartorius filtration system and then the filter content resuspended in sterile normal saline then took 5 µl and cultured on the above mentioned agar, incubated at 37°C for 48 hr<sup>[20-22]</sup>.

### Dosing:

The experiment includes (20) Rats (Albino Rats ) of males and females have (same of the age ,weight and conditions ) treated from Al-Yohedia stream by drinking at need for two months whereas control groups were treated with (RO) tap water and divided into five groups: first group dosed with site 1 sewage samples , second group dosed with site 2 sewage samples , third group dosed with site 3 sewage samples , forth group dosed with site 4 sewage samples and fifth group dosed with tap water was control and after complete the period of dosing rats anatomized by anesthesing rats with chloroform and then with drawing 5ml blood from heart area by syringe for detection molecular biomarker by comet assay and take tissue (liver) for histopathology and immunohistochemistry (IHC) tests<sup>[23]</sup>.

### Comet Assay:

This assay has been done by Comet assay Kit, Maltgene-Italy (Single cell gel Cat. No. 0905-050- K) according to (Steinert, 1996)<sup>[24]</sup>.

**Immunohistochemistry (IHC):**

Immunohistochemistry assay include using of specific monooclonal / polyclonal antibody and detection kit directed toward this antibody. In this study we used Anti-8 Hydroxyguanosine antibody [N45.1] (8-OHDG) (cat. # ab48508, Abcam /England). The immunohistochemistry detection kit is a reliable and convenient tool to identify specified gene expression on tissues. A biotinylated, cross – adsorbed and affinity purified primary anti-mouse and rabbit specific HRP/ DAB is used to detect primary antibody –antigen complexes adhered to a glass microscope slide, following reaction with an enhanced detection reagent. This kit is compatible with mouse and rabbit specific HRP/DAB secondary antibodies and can be used for paraffin – embedded tissue sections (Cat. Number: Ab80436, Abcam, England).

**Histopathological Study:**

Specimen with dimension 1x1x1 cm was taken from inside organ together with liver. The tissue have been fastened in (10%) buffer formaldehyde re at this time later removing wards 72 hr of the fixation , specimen was washed with tap water after which processing was robotically carried out with a collection of upgrading alcoholic focus from (70%) to genuine (100%) for two hr in every focus to eliminate water from the tissue , then clearance was executed by xylol , then the specimen was

infiltrated with Simi-liquid paraffin wax at( 58°C) on two phases then blocks of specimen was made with paraffin wax and sectioned by rotary microtome at 6 µm for tissue , tissue was stained with haematoxylin and eosin and the Histopathological vary was noticed beneath mild microscope [25].

**Quantitation of Dissolved Heavy Metals:**

Concentrated hydrochloric acid (5 ml) was added to 1 L of the waste water filtrate and concentrated by oven (800C) until evaporate to 100 ml and then measured by Flame Atomic absorption - spectrophotometer model (7000) Aa type (Shimadzu/Japan) was used to determine the concentrations (mg/L) of the elements [26].

**Results and Discussion:**

The results of dissolved heavy metals concentration revealed high Ni concentration (Mean±SD of four sites were 119.675±0.778 mg/L), high Mn concentration (Mean±SD of four sites were 108.2±1.095 mg/L), high Fe concentration (Mean±SD of four sites were 119.675±0.740.475±1.228 mg/L), high Cd concentration (Mean±SD of four sites were 13.75±1mg/L), high Cu concentration (Mean±SD of four sites were 5±0.755mg/L) with no significant differences in concentration among four sites for Ni and Cu while difference noted among Mn, Fe and Cd (table 1). All values were higher that standard permissible limits of WHO [27-30].

**Table (1): Concentrations of dissolved heavy metals of municipal wastewater.**

Dissolved mg /L	Site1	Site2	Site3	Site4	Mean±SD
Ni(Nickel)	120.7 ±0.1 a	119±1 a	119±0.01 a	120±2 a	119.675±0.778
Mn(Manganese)	116.6±2.08 d	100.1±0.1 a	110.2±0.2 c	106±2 b	108.2±1.095
Fe(Iron)	30.6 ±0.4 a	55±3 c	32 ±0.01 a	44.3±1.5 b	40.475±1.228
Cd(Cadmium)	12 ±1 a	12±0.01 a	16±2 b	15±1 b	13.75±1
Cu(copper)	6±0.01 a	5 ± 2 a	4± 1 a	5±0.01 a	5±0.755
Pb(Lead)	2.7±0.6 a	3 ± 2 a	3±2 a	0.8 ± 0.01 a	2.375±1.152

p&lt;0.05

The main sources of lead contamination are industrial threw battery in sewage, run off from contaminated land areas, atmospheric fall out and sewage effluents. The sources of heavy metal also the reason in the high concentration of nickle in Al-Yohedia stream may be attribute to the high numbers of small engine oil exchange and electricity station near to stream, disposal of tires of cars in the stream, generator located at the side of stream .The exchanged oil and waste of generators delivered directly to stream in addition to the nickel not use and not consume by microorganism [31]. Cd was classifies as a first category of carcinogenic factors in human cancers because cadmium ions have a configuration of electrons analogous. Pb responsible for oxidative stress and genotoxicity [32-35].

Results of microbial study revealed that the Mean±SD of CFU/ml were  $1.97 \times 10^{10} \pm 0.543$  and the microbes were *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *V. cholerae*, *V. parahaemolyticus*, *S. aureus*, *E. faecalis*, *C. albicans*, *C. krusei* and *C. glabrata* with MAR index ranged from (0-1) (table 2). The results agreed with Yang et al., (2009) [36] who found that the clinical isolate of *E. coli*, *K. pneumoniae*, *S. aureus* and *P. aeruginosa* were common among sewage water. Concern presence of *Candida* spp. it supported by Filipkowska et al., (2008) [37], Biedunkiewicz and Ozimek, (2009) [38] and Korzeniewska et al., (2011) [39] whom found the different species of *Candida* in municipal wastewater. It is important to state that recovering mentioned microbes from high concentration of heavy metals waste water reveal their ability to live with such toxic concentrations may be due to their efflux pump or plasmid coding genes. The heavy metals and antibiotic resistance were closely related and may be carried on same plasmid [40-44].

**Table (2): Microbes with resistance phenotypes and MAR index.**

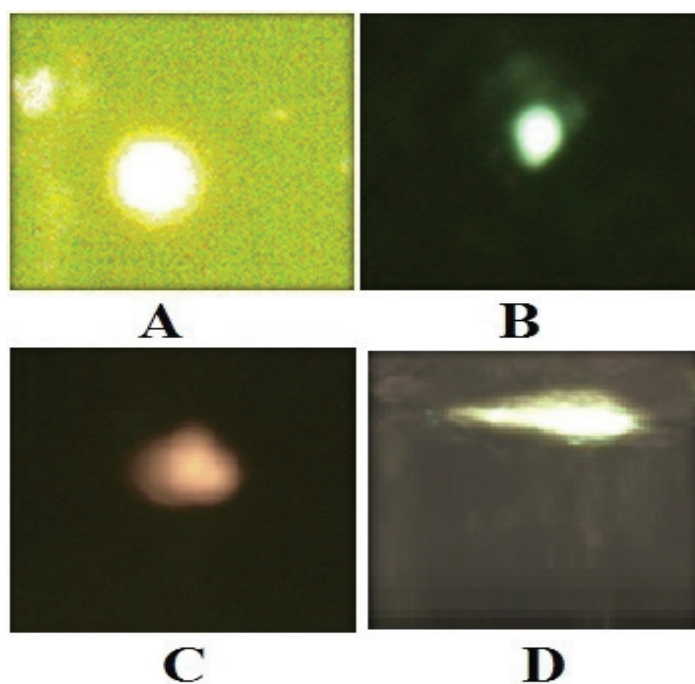
Pathogen	Resistance Phenotype	MAR index
<i>E. coli</i>	AmoxiclavR/CefotaximeR	0.22
<i>K. pneumoniae</i>	AmoxiclavR/CeftazidimeR	0.22
<i>P. aeruginosa</i>	AmoxiclavR/CefotaximeR/CeftazidimeR/AmikacinR	0.44
<i>V. cholerae</i>	NON	0
<i>S. aureus</i>	ClarithromycinR	0.1
<i>E. faecalis</i>	NON	0
<i>C. albicans</i>	KetoconazoleR/MiconazoleR	0.33
<i>C. krusei</i>	KetoconazoleR/MiconazoleR/EconazoleR/FluconazoleR /ItraconazoleR/ClotrimazoleR	1
<i>C. glabrata</i>	KetoconazoleR/MiconazoleR/EconazoleR/FluconazoleR /ItraconazoleR	0.83

DNA damage results using comet assay for revealed that high level of damage to DNA of rat dosed with waste water compared with those dosed with fresh water in both tail length ( $11.35 \pm 2.1$ ,  $2.7 \pm 0.9$ ) and tail moment ( $1.193 \pm 0.11$ ,  $0.2 \pm 0.1$ ) respectively (table 3).

DNA damage based on the comparison with the control according to the pictures that were taken DNA damage can be classified into three classes (class 1, no damage; class 2, low damage; class 3, medium damage and class 4, high damage) as shown in figures (1). The observed DNA damage may attributed to heavy metals which have a role in high percentage of DNA damage and ability to produce bulky DNA adducts. The heavy metals can produce chemical or physical modification to DNA measured by means of DNA fragmentation [45].

**Table (3): Comet Assay Parameter among rat dosed with waste and fresh water.**

Comet Parameter	Mean±SD	
	Rat dosed with waste water	Rat dosed with fresh water
Tail Length (µm)	11.35±2.1	2.7±0.9
Tail Moment (%)	1.193±0.11	0.2±0.1



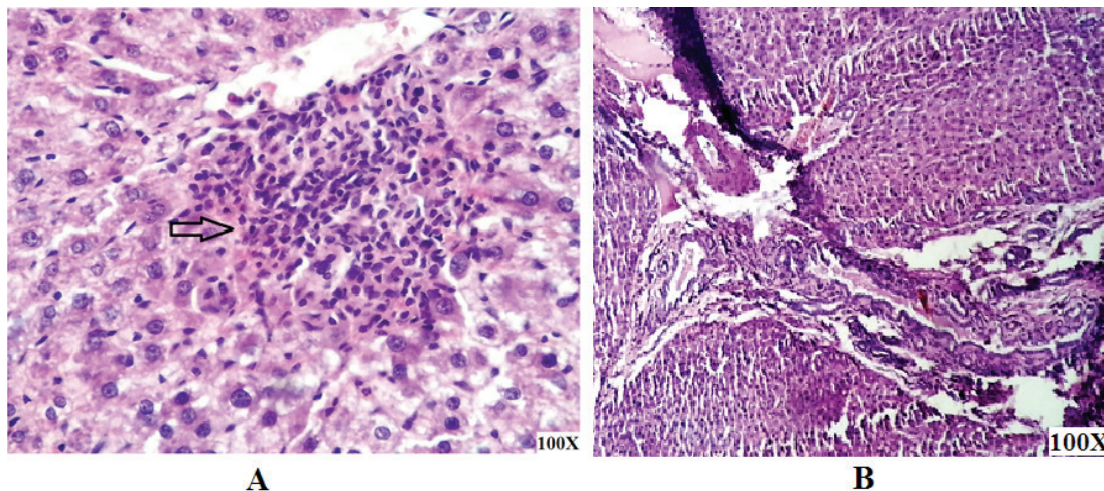
**Figure (1): Classes of DNA damage in rat sample (blood) oral administrated from wastewater of four sites according to comet assay: Class (A) Control, Class (B) low damage, Class (C) Medium damage, Class (D) High damage.**

Immunohistochemistry (IHC) assay was used to detect of 8-OHdG among liver tissues of rat dosed with waste water (study) and fresh water (control) and the results revealed strong signal (high expression) in all liver tissues of rat dosed with waste water (study) compared with those dosed with fresh water (control) whose gave negative results (figure 3). High expression of 8-OHdG revealed high oxidative stress caused by

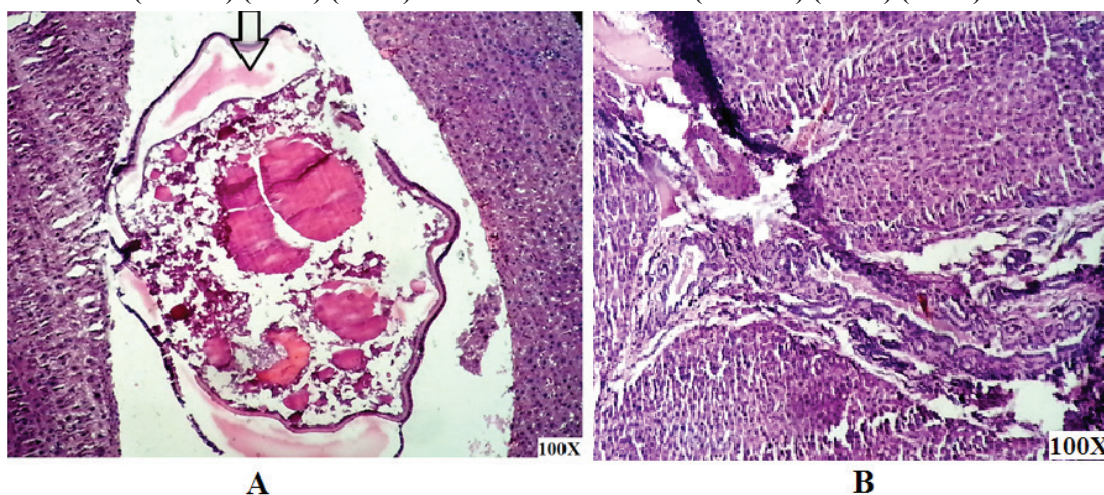
pollutants especially heavy metals like Cd and Pb. liver tissues used to understanding biotransformation and toxicity in municipal wastewater, this was agreement with Herman and Geraldine, (2009)<sup>[46]</sup> who considered that the liver is one of the target organs affected by lead toxicity owing its storage in the liver after lead exposure. Also it is organ of detoxification <sup>[47,48]</sup>.

**Figure (3): Infiltrative rats liver tissues showing the results of immunohistochemical staining of 8-OHDG protein over expression using biotinylated -labeled anti-8-OHDG protein antibody, stained by DAB-chromogen (Brown) and counter stained by Mayer's haematoxylin (Blue): A. Positive 8OHDG –IHC reaction with high score and strong signal intensity (10X) B. Liver tissues with negative 8-OHDG-IHC reactions (10X)**

Histopathological results of eat dosed with waste water compared with those dosed with fresh water (control) were observed after 60 days of dosing . the results displayed that the control was normal where mild chronic hepatitis showed in samples of site 1, 2, 3 while site4 had moderate chronic hepatitis and *Fasciola hepatica* (figure 4 and 5). Many studies of mammalian tissues noted that the histopathological alterations induced by different heavy metals in the tissues of liver organ. Human and animal wastewaters play role important as sources of parasitic pathogens infecting human [49,50].



**Figure (4): Show the histopathology of rat Liver sections after 60 days of dosage with wastewater: A:Show liver tissue with moderate chronic hepatitis, intralobular and periportal chronic inflammatory cell infiltrate (arrows) (100X) (H&E). B:Show normal Liver (Control) (100X) (H&E).**



**Figure(5): Show the histopathology of rat liver sections after 60 days of dosage with wastewater: A: Show the liver tissue with Fasciola spp. (Fasciolosis) (arrow) (100X) (H&E). B: Show normal Liver (Control) (100X) (H&E).**

**Conflict of Interest:** we declare that there is conflict of interest

**Ethical Approval:** the research approved by scientific and ethical committee at our department

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