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Toxicity Effect of Olive Mill Wastewater on fish species (common carp) *Cyprinus carpio*

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Abstract
The study was conducted in the Laboratory of Marine Sciences and the Aquatic Environment during 2017, to study the effect of olive mill wastewater on the fish species, common carp *Cyprinuscarpio*, which were selected as a vital indicator for toxicity tests.The experimental fish were selected with a standard length of 10 ± 2 cm and distributed on glass containers (70 x 40 x 40 cm) with 10 individuals per container, The studied fish were subjected to four different concentrations of OMW, 2.5%, 5% and 10% (volume / volume), in addition to the control. After that, the mortality rate of fish was recorded 24 hours after the addition of OMW, Then the results were analyzed by SPSS program to calculate the LC_{50}. The results showed that the mortality rate of LC_{50} of the studied species obtained at the concentration level of 1.48% in the water body. The value of the acute toxicity unit was ATU (54.05). The results also showed that the safe concentration of OMW disposal should be very low ≤0.14% (volume / volume). These results indicate that OMW has a acute toxicity to the fish.

1. Introduction
Environmental problems have become the focus of attention in most countries of the world. These problems have emerged as a result of the increasing frequency of improper use of environmental resources. They have compromised the ability of ecosystems to regenerate spontaneously and have upset the natural balance of these systems. One of these problems in Syria is the wastewater of olive oil extraction from olive fruits, which is called **"Olive Mill Wastewater" (OMW)**. Which is disposed of directly without any treatment into the nearby areas, leading to pollution of soil, groundwater, rivers, lakes and even seas, And the threat of aquatic life and the poisoning of plant, animal and organisms because of its contents of toxic chemicals. The olive yield in Syria is an important yield, and the area cultivated with olive trees is more than (60%) of the total area of fruit trees in the country [1]. Also, olive is considered one of the main yield in Lattakia Governorate. Where its cultivated area for the year 2016 is about 47318 ha, and its oil production for the same year is estimated at 33000 tons, producing more than 82500 m^3 of olive mill wastewater (OMW) [1]. Olive mill wastewater (OMW) causes high pollution if it is disposed of directly in the environment without treatment due to its high load of some organic compounds. It also contains many chemical compounds, including sugars, tannins, poly phenols, alcohol, pectin, fat, ...etc, [2].

Olive mill wastewater (OMW) contains a large proportions of simple sugars, which will increase the number of microorganisms that use these sugars as a source to build their bodies, and this leads to an increase in the consumption of oxygen and reduces its available amount to the organisms, causing a defect in the entire ecosystem, Similarly, high concentrations of phosphorus in (OMW) can affect the ecological balance of water, encouraging and accelerating the growth of algae, increasing the chances of food enrichment, and destroying the ecological balance in the natural waters, Phosphorus also moves to high levels across the food chain, moving to plants, including invertebrates, fish and then to waterfowl [3].

Due to the high organic load and the high content of phenolic compounds, (OMW) shows high biochemical demand for oxygen and chemical demand for oxygen (BOD / COD) values, which adversely affect the life of aquatic organisms [4]. Their values are about 200-400 times higher than those found in municipal wastewater [5]. The importance of the experiment show through the fact that (OMW) is considered one of the most important pollutants for fresh water in the Syrian coast, which is disposed of directly without any treatment in the water bodies, which adversely affects aquatic organisms, C. *Carpio*, is considered one of the most important fish species spread in Syrian fresh water for several reasons:

1- it is the most widespread Species.
2- it is considered to be one of the desired fish by the population and of high economic importance.
3- it is the most resistant to environmental stresses [6].

Materials and methods:

• Olive mill wastewater samples:
Composite samples of (OMW) were collected during the 2017 season of olive oil production, from a group of Full automatic (centrifugal) olive mills in Lattakia Governorate, The sample of OMW was transferred to the Marine Science and Aquatic Environment Laboratory (Tishreen University), and stored at 4 ° C until use.

• Physical, chemical and biological characteristics of (OMW):
The bio-phyisco-chemical characteristics of (OMW) were determined according to the standard analytical methods [7].
All the following measurements were carried out in the laboratories of Water Resources Directorate (Lattakia):
pH; - Bio-oxygen demand (BOD5); Chemical Oxygen Demand (COD); - Total nitrogen; Total phosphorus; Poly-phenols.

• Test organisms:
The experimental fishes were brought from Al-sein Fisheries farm (Banias-Tartous). The healthy fishes, with a standard length of 14 ± 2 cm were selected and then transported in 20 liter plastic bags (one third of fresh water and two thirds of air) to the Marine Science and Aquatic Environment Laboratory at Tishreen University. They were placed in a 250 litter container for two hours to pre-adapt with the laboratory water temperature, Then the individuals were distributed in the experimental ponds (12 containers) (70 x 40 x 40 cm) with an average of 10 individuals in each container , and left grow up for a period of 15 days to adapt to the new environment.

• **Test procedures:**

The test design consisted of three concentrations levels (OMW) 2.5%, 5% and 10% (volume/volume) in the natural environmental conditions in glass tanks water. (OMW) was added to the glass tanks according to the above mentioned concentrations, fish were tested in 3 replicates per concentration, in addition to three glass tanks without (OMW) as a control. After (OMW) addition to the glass tanks water, test fish were not fed during the test with continuous ventilation system. After 24 hours from (OMW) additions. All treatments were checked for mortality after 24 h and dead fish were counted, along with measuring temperature and pH values and dissolved oxygen.

• **Data analysis:**

The number of dead fish at each concentration was converted to percentage and the mean of each replicate was computed and compared with the control. Data were evaluated by one way analyses of variance (ANOVA) and multiple comparison tests by less significant difference method (LSD). Statistical analysis was performed using the statistical program (SPSS) for windows (version 19). The half lethal concentration LC_{50} (ie, the concentration level which half of the experimental fish dies at) values and their respective confidence intervals (95%) were calculated based on the pooled data set for a given test fish by using (SPSS v.19). The acute toxicity unit (ATU) and Safe concentration level (SCL) were calculated by the formulas indicated by Roopadevi and Somashekar, (2012) [8]:

$$ATU = 100/LC_{50}\%$$

$$SCL = LC_{50}\% \times 0.1$$

The acute toxicity unit was classified according to the acute toxicity classification system reported by Pearson [9].

Results and discussion:

Table (1) shows that OMW is slightly acidic with high BOD, COD, nitrogen and polyphenols content, but as compared to literature values, all measured values of each parameter tested were within the range of previously reported values for OMWs [10] (See table 1).

Table (1): Some bio-physio-chemical characteristics of the OMW:

Component	Concentration
pH	5.2
COD	95500(mg /L)
BOD	55500(mg /L)
Total nitrogen	855(mg /L)
Total phosphorus	150 (mg /L)
Total polyphenols	5100 (mg /L)

Table (2) shows that the average mortality percentage of fish at different concentration of (OMW) and this results were tested by ANOVA test. The P-value was found less than (0.05). Thus, there are significant differences between the mean of the three concentrations of test.(see table 2).

Table (2): Average mortality percentages of fish at three concentrations: 2.5, 5.0, 10% (v/v) of OMW and control after 24 h exposure period:

OMW Concentration (Volume / Volume)%	Mortality (%)
Control (0%)	0%
2.5%	76.6%
5%	93.3%
10%	100%

The multiple comparison tests of the three values of (OMW) were studied by applying the least significant difference method (LSD). The LSD method results shown that there are significant differences at 5% significant level between the averages of different treatments where the value of P-value is less than 0.05. Also, There is no significant difference between the two treatments (5% and 10%) where P-value was greater than 0.05. It is apparent from the present study that the OMW was toxic to all test fish and the extent of toxicity as demonstrated by the mortality percentage became progressively higher with increasing OMW concentrations. On the other hand, the mortality in the controls of all fish tested was 0%; demonstrating that the holding facilities, water and handling techniques were acceptable for the toxicity test

The death of test fish could be attributed to the lethal action of OMW constituents that resulted in alterations in physiological processes at the cellular levels as well as changes in biochemical process related to cellular metabolic pathway. For example, OMW was found to interfere with mitochondrial bioenergetics such as mitochondrial respiration mitochondrial membrane potential and respiratory complexes which is known to be a part of the process of cell injury [11] Which led to abnormalities in nuclei of cells and DNA damage. Moreover, OMW was reported to have a large number of toxic chemicals such as tannins and phenolic compounds [12], as well as catechol and hydroxytyrosol[13], to which the mortality of aquatic organisms could be attributed.

The mortality of aquatic organisms could also be affected by other factors such as COD, BOD, low molecular weight phenolic compounds, free fatty acids, as well as phenoxy radicals [14]. In addition to the pH value, dissolved oxygen, solids, organic matter, temperature etc. when present beyond their maximum tolerable levels [15].

Data in the table (3) shows that the concentration of dissolved oxygen has decreased after the addition of OMW to below the appropriate concentration for fish (5 mg / L) [16] (see table 3). This explains why the fish is inhaled at the surface of the water as a final solution to obtain oxygen ,Where the presence of oxygen within its inappropriate levels will lead to the failure to maintain the quality of breathing and thus metabolism and utilization of nutrients, which it turn reflects negatively on the rates of growth and reproduction. In addition, table (3) shows a decrease of pH values from the permissible limits of fish (6.5-9) [16]. In acidic water conditions, fish lose their appetite for food, the ability to grow, resist diseases and toxic substances, and severe pH negatively affects the growth and fish reproduction, resulting in widespread mortality of fish and also affects the physiology of the general body and physiology, especially reproduction [17]. The acidity of the OMW and its low pH value are due to the presence of many fatty acids such as oleic, palmitic, palmitolic, linoleic and linolenic acid [18]. The LC_{50} values of

OMW along with their respective 95% confidence limits (lower and upper confidence limits) for test fish are presented in Table 4

Table (3) Values of dissolved oxygen and PH for experimental treatments before and after the addition of OMW:

	Dissolved oxygen (mg / l)		PH	
	Before adding	After adding	Before adding	After adding
Control (0%)	20	19.5	.74	7.1
2.5%	19.5	4.9	7.7	6.4
5%	20.2	3.4	7.5	5.9
10%	19.3	2.6	7.2	5.4

Table (4): LC_{50} values and 95% lower and upper confidence limits, for OMW after 24 h interval for the fish

	%95Confidence limits(%)		
LC_{50} %	Lower	Upper	chi-square
1.48	0.83	1.94	0.222

The table (4) also shows that, the calculated chi-square values were less than the tabular value (5.991) at 5% level with 2 degrees of freedom. This indicates that, the chi-square test for heterogeneity was not significant ($p > 0.05$) and thus, the Probit Analysis appeared to be appropriate for this data (Table 4). In this work we found that the LC_{50} value of the experimental fish was found within 24 hours after adding OMW 1.48%. Previous studies have shown that LC_{50} values vary with the variation of organisms species. For example, this value was for species *B. plicatilis* 3.3%, *A. salina* 5.1% ,*Mytilus SP* 6.5% and finally species *B. amphitrite* 7.1% [19].The LC_{50} value for small crustaceans of *Thamnocephalus platyurus* ranged from 0.73 to 12.54% [12]. The difference in sensitivity among the different species to the OMW may be due to the difference in their ability to metabolize, excrete and store of the toxic constituents of OMW and/or the difference in the transportation of such constituents into the site of action [19]. It was found in this work, that the fish have a LC_{50} value lower than most of other aquatic organisms, which means that the fish species *Cyprinus carpio* are the most sensitive to OMW. This could be explained by the fact that fish live in a relatively pollution-free environment, so they are sensitive to pollution. Some studies have shown that aquatic organisms living in polluted environments have high LC_{50} values compared to those living in clean environments [20]. Some studies have shown that the values of LC_{50} can vary between species and within the same species according to different experimental conditions. Therefore, comparing this indicator between species or even within the same species in different experimental conditions is impractical [21]. According to the acute hazard classification system reported by Persoone *et al.*, (2003)

[9], acute toxicity units (ATU) greater than or equal to 10 and less than 100 (10 ≤ ATU< 100) is considered highly toxic.

In the present study, the acute toxicity unit obtained for OMW (ATU) 67.56, and therefore, OMW can be considered to have a high acute toxicity to *C.carpio*. On the other hand, the safe concentration of OMW to be discharged into aquatic water bodies which is highly useful in establishing limits of acceptability by the aquatic organisms was found to be very low 0.14% (v / v), which is very useful in determining the appropriate limits of aquatic organisms.

Conclusion:

The acute experience of OMW on the fish species Cyprinus carpio showed that OMW is highly toxic to fish and other aquatic organisms if directly disposed into the water bodies without treatment.

Therefore, it is necessary to take an appropriate measure to prevent the access of peat water directly to the bodies of water through the monitoring of olive process and the necessary treatment of OMW before the discharge, and further studies should be done on the toxicity of OMW to other living components of the water environment.

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