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Acute Toxicity of Olive Mill Wastewaters from the Gaza Strip to Four Marine Invertebrates

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Abstract

Background: The Olive Mill Wastewater (OMW) that results from olive oil production processes, is a potential toxic pollutant, adversely affecting the fauna of aquatic ecosystem. Due to the absence of implementation of stringent rules and regulations to control the disposal of industrial wastewaters into the environment, most of the wastewaters generated from the different olive mills in the Gaza Strip are usually discharged into sewer systems or into Wadi Gaza which finally reach the Mediterranean Sea.

Objective: The present study aims to evaluate the acute toxicity of OMW to four marine invertebrates; *Artemia salina*, *Balanus amphitrite*, *Brachionus plicatilis* and *Mytilus* sp..

Materials and methods: Composite samples of OMW were collected from an olive mill near Gaza City and the physicochemical characteristics such as pH, EC, BOD, COD and total nitrogen were analyzed. The test organisms were exposed to four concentrations of OMW, 1.25, 2.5, 5 and 10% (v/v) under static conditions. Mortality data were recorded after 24 h and analyzed using U.S. EPA Probit analysis software to calculate LC_{50} values.

Results: The results of the physicochemical characteristics of OMWs were found to fall within the range of the reported literature data. In the terms of median lethal concentration (LC_{50}), the order of sensitivity of tested organisms was *B. plicatilis* > *A. salina* > *Mytilus* sp.> *B. amphitrite* with estimated LC_{50} values of 3.3%, 5.1%; 6.5% and 7.1% (v/v) respectively. Based on derived 24h LC_{50} values, OMW appears to be highly toxic to tested organisms with acute toxicity units (ATUs) ranged from 14 to 30. The safe dischargeable concentration of OMW was found to be very low i.e. ≤ 0.710% (v/v).

Conclusion: Results indicated that OMW is highly toxic to marine invertebrates and may pose relatively serious hazards to receiving waters; accordingly, it should be treated before disposing to aquatic environments.

Keywords: Olive mill wastewaters, Acute toxicity, Marine invertebrates, Gaza Strip.

السمية الحادة لمخلفات الزيتون السائلة من قطاع غزة لأربع كائنات لافقارية بحرية

ملخص : تُعتبر مخلفات الزيتون السائلة والناتجة من عمليات عصر الزيتون من المخلفات السامة التي قد تؤثر سلبيا على الكائنات الحيوانية في النظم البيئية المائية . نظرًا لعدم تطبيق قوانيين صارمة

تضبط عملية التخلص من هذه المخلفات فإن معظم المياة العادمة الناتجة من المعاصر المختلفة في قطاع غزة عادة ما يتم التخلص منها بالقائها في انظمة الصرف الصحي او في وادي غزة و التي ينتهي بها المطاف في البحر الأبيض المتوسط. تم جمع عينات لمخلفات الزيتون السائلة بهدف تقييم سميتها الحادة على اربع كائنات مائية لافقارية و هى *Artemia salina* و *Balanus amphitrite* و *Brachionus plicatilis* و *Mytilus* sp.. تم قياس بعض الصفات الفيزيائية والكيميائية للمخلفات السائلة مثل pH و BOD و COD و النيتروجين الكلي ووجدت بأنها تقع ضمن المدى المذكور ضمن بيانات الدراسات السابقة في هذا المجال . تم تعريض كائنات الإختبار السابق ذكرها لأربع تراكيز مختلفة من مخلفات الزيتون السائلة وهى 1.25 و 2.5 و 5 و 10% (حجم/حجم) ومن ثم تسجيل الوفيات بعد 24 ساعة و تحليل النتائج باستخدام برنامج التحليل الاحصائي "البروبيت" لحساب متوسط التركيز المميت (LC_{50}). بناء على نتائج حساب متوسط التركيز المميت فقد كان ترتيب كائنات الاختبار تبعا لحساسيتها كما يلي: *Mytilus* sp. < *A. salina* < *B. plicatilis* < *B. amphitrite* وقد كانت قيمها على التوالي هى 3.3 و 5.1 و 6.5 و 7.1 (حجم/حجم). بناء على هذه النتائج فان مخلفات الزيتون السائلة اظهرت سمية عالية تجاه كائنات الاختبار وقد تراوحت وحدات السمية الحادة (ATUs) من 14 الى 30. و قد وجد بأن التركيز الآمن للتخلص من مخلفات الزيتون السائلة يجب ان يكون قليل جدا أي $\geq$ 0.710 (حجم/حجم). دللت نتائج السمية الحادة بأن مخلفات الزيتون السائلة تمتلك سمية عالية تجاه الكائنات اللافقارية البحرية و من الممكن ان تشكل مخاطر ضارة على الاجسام المائية المستقبلة لها وبناء على ذلك فانه يجب معالجتها قبل القائها في البيئات المائية.

الكلمات المفتاحية: مخلفات الزيتون السائلة، السمية الحادة، اللافقاريات البحرية، قطاع غزة

1. Introduction

Aquatic systems are usually exposed to a number of pollutants generating from industries, sewage treatment plants, drainage from urban and agricultural areas. Industrial effluents are possibly the most important source of contaminants to aquatic environment [1].

Olive mill wastewater (OMW), the liquid by-product, generated by the olive oil extraction process is the main waste product of this industry. Approximately, 1.8×10^6 tons per year of olive oil are generated throughout the world, the majority (98 per cent) of it is produced in the Mediterranean basin [2]. It is reported that OMW resulting from the production process surpasses 30×10^6 m^3 per year in the Mediterranean region [3].

During the olive mill season which extends from early October to late December, the Gaza Strip produced an average of 838 tons of olive oil, from about 4760 ton of pressed olive, through the period of 1995-2012 (Table 1). Unfortunately, there is no data available concerning the total amount of

OMWs (known in Palestine as Zibar) produced in the Gaza Strip. An estimate however can be performed based on data from some Palestinian cities at West Bank such as Hebron, Nablus and Jenine, where 1 ton of processed olives produces approximately 1.7 m^3 of wastewater [4]. Considering similar situation, and based on data obtained from the Palestinian Central Bureau of Statistics [5] the amount of OMW produced annually in the Gaza Strip as a result of the olive mill activities ranges from 1598 to over 18835 m^3 (Table 1).

Table 1 Summary of quantity of olive pressed (metric ton), oil extracted (metric ton) and OMW (m^3) produced in the Gaza Strip for the years 1995-2012 [5].

	Quantity of olive pressed (metric ton)	**Quantity of oil extracted** (metric ton)	**OMW (olive pressed ×1.7)** (m^3)
Total	85665.50	15097.50	145631.35
Average	4759.19	838.75	8090.63
Max	11079.80	1851.00	18835.66
Min	940.00	189.00	1598.00

Despite being recognized as environmentally hazardous material, the common practice in the Gaza Strip is to dispose OMWs into sewerage systems [5,6] or, though in lesser extent, into Wadi Gaza [7]. In both cases, OMW and/or their degradation products will eventually reach the Mediterranean Sea, contaminating the coastal waters and may cause adverse effects on marine organisms, thus, deteriorating the very limited natural resources of the Gaza Strip.

The environmental implications from the uncontrolled disposal of OMW has been recognized for a long time and are mainly related to their high organic loads [8], high amounts of organic acids [9] and phenolic compounds [9,10] and their toxicity to several groups of organisms [10-12].

In many countries of the world, physical and chemical parameters are routinely used to evaluate the quality of industrial effluent. These parameters alone however, do not allow evaluation of the risks associated with the discharge of such effluents into marine environments or to examine their toxic effects on marine life inhabiting these environments. Acute toxicity test on the other hand, is a relatively simple laboratory bioassay that quantify the lethal concentrations of a pollutant on a particular species and used in some countries to evaluate the potential harmful effect of industrial wastewater effluents on the aquatic life. The calculated value, termed as median lethal concentration (LC_{50}) corresponds to the concentration of a pollutant that provokes death in 50% of an experimental organisms after a given exposure time, generally 24-96 hours.

A large number of test organisms, including, microorganisms, invertebrates, and vertebrates have been proposed to be used in bioassay tests. To be of practical use in such bioassays, a candidate organism, should fulfill many selection criteria which have been defined by many authors and including the wide geographic distribution, sensitivity to potential contaminants, easiness of collection from the field (i.e. abundant), amenability to routine maintenance, culture and rearing in the laboratory [13] as well as the short life-cycle, large offspring production and the existence of a considerable amount of information about these organisms.

Although a considerable volume of information is available on the toxicity of various industrial effluents, not much work has been done on the acute toxicity of OMW to marine invertebrates [10, 14, 15]. Hence, in the present study four marine invertebrates representing three phyla; the Arthropoda, brine shrimp (*Artemia salina*) and the acorn barnacle (*Balanus amphitrite*), the Mollusca, mussel (*Mytilus* sp.) and the Rotifera, rotifer (*Brachionus plicatilis*) were chosen as test organisms to evaluate the acute toxicity of OMW. These invertebrates were selected as study species for bioassay because they meet most of the requirements for a model test species. Furthermore, the mussel, *Mytilus* sp. and the barnacle, *B. amphitrite* are representative species of marine invertebrate communities of Mediterranean rocky substrata in Palestinian coasts -as well as other parts of the world- thus, they are assumed to reflect the local environmental status and the impact on the environment caused by human activities.

2. Materials and Methods

2.1. OMW Sampling

Composite samples of OMWs were collected at different times during 2012–2013 extraction season from a full automatic (centrifugal) olive mill located near Gaza City. The samples were transferred to our laboratory (Department of Biology, The Islamic University of Gaza), where they split after homogenization into several 2L clean plastic bottles, and stored frozen at -20 °C until use for the toxicity tests.

2.2. Physicochemical analysis of OMW

The physicochemical characteristics of OMWs were determined according to standard analytical methods. All measurements were conducted in duplicate. The **pH** and **electrical conductivity** (EC) were measured by using a properly calibrated benchtop pH meter (Hanina Instruments, Italy) and conductivity meter (Bookel, Korea) respectively according to manufacturers' instructions. The **Biochemical Oxygen Demand** (BOD_5) was determined with the manometric method using a respirometer OXITOP (WTW, Germany). The potassium permanganate method according to the Standard Methods for Examination of Water and Wastewater-18th Edition [16] was used for the determination of **Chemical Oxygen Demand** (COD). **Total kjeldahl nitrogen** content was determined with the use of DK6 digestion unit and UDK142 distillation unit (VELP Scientifica, Italy).

2.3. Seawater

All experiments were performed using Mediterranean seawater collected from unpolluted site at the coast near Gaza city and filtered through Whatman GF/C glass microfiber filters using a vacuum pump. Salinity was adjusted to 35‰ (parts per thousand) and kept in dark containers for later use.

2.4. Test organisms

2.4.1. *Artemia salina*

One day before the beginning of toxicity test, commercially available brine shrimp cysts (Great Salt Lake Artemia cysts, Artemia International LLC., Fairview, Texas, USA) were hatched in sea-water (35‰) at room temperature (~25°C) under conditions of continuous illumination and aeration. Freshly hatched individuals, so called nauplii, were used for testing. After exposure to test concentrations, organisms were checked for death and those with no response to stimuli at all were considered dead.

2.4.2. *Brachionus plicatilis*

The rotifer, *B. plicatilis* was imported from Egypt in 2010 for the purpose of establishing a marine fish farm at Dier Elbalah city. Since then, this strain has been cultured continuously in our labs. Rotifer cultures were carried out using seawater at 25‰ salinity and using the green unicellular algae, *Nanochloropsis oculata* as exclusive food. Rotifers were transferred every four days to fresh medium to maintain the cultures in the logarithmic phase of the amictic growth. To minimize age variations, a synchronized generation of rotifers was used for the toxicity test. This was attained by transferring amictic females to fresh media supplemented with *N. oculata* and allowed to lay eggs for 4-6 hours. The females were then removed and the eggs were left to hatch overnight at 25ºC. Newly hatched rotifers (neonates) were collected and used in the test. Similar to *Artemia*, the criterion for determining death after exposure was the absence of response when the animals were stimulated.

2.4.3. *Mytilus* sp.

Mussels, *Mytilus* sp. of a comparable size (1.5 – 2.0 cm shell length) were collected from natural coastal populations living on hard substrates at Gaza fishing port (Figure 1). Mussels were carefully removed from their byssal attachment and brought back to the laboratory. The mussels were cleaned and maintained at room temperature in aerated, filtered seawater for 3 days before the experiments for acclimatization. Organisms passed the acclimatization period were used in the test. After exposure to test concentrations, mussels that gaped wide were considered dead.

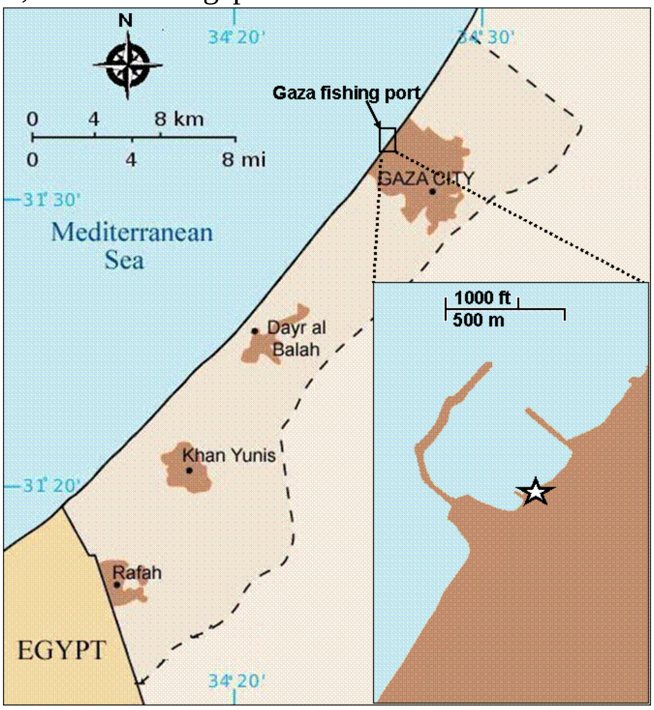

Figure. 1. Locations of Gaza fishing port near Gaza City, Gaza Strip, Palestine.

2.4.4. *Balanus amphitrite*

Small pieces of stones and cobbles covered with large, living barnacle shells were collected from Gaza fishing port. Their surfaces were cleaned by brushing smoothly to eliminate debris and any other living things. They were acclimated in the same way as described for mussels. The effect of OMW was evaluated by immersing barnacle (while attached to cobbles) in 100-ml beakers filled with the test concentrations. Only individuals with active cirri movement were used in the experiments. At the end of the exposure period the barnacles were rinsed thoroughly with clean seawater and transferred to beaker filled with filtered and aerated seawater for recovery. Individuals failed to resume their cirri movements after 2 hours were considered dead.

2.5. Test procedure

The OMW was prepared for exposures by defrosting a sample and warming it to room temperature (~25°C). The test design consisted of four concentrations of OMW: 1.25%, 2.5%, 5.0% and 10% (v/v). The different concentrations were made by using clean, filtered seawater. Prior to the exposure, all test solutions were adjusted to 35‰ salinity (except rotifer, 25‰) and pH 8. Groups of experimental animals, each consisting of 10 individuals, were selected at random and placed into test vessels filled with tested concentrations. Glass beakers of 100-ml capacity were used as test vessels for barnacles and mussels, while 55 mm diameter Petri dishes were used for *B. plicatilis* and *A. salina*. Controls were treated in the same way but without adding of OMW to the seawater. All handling processes of both *B. plicatilis* and *A. salina* were made by using fine pipettes under a stereomicroscope. To minimize the dilution of the test solution in the actual test vessels, brine shrimps and rotifers were first collected into beakers filled with given concentrations of tested OMW. Organisms were tested in 3-6 replicates per group, resulting in a total of 30-60 individuals for each concentration. All experiments were made in temperature-controlled cabinet at a temperature of 25 ± 1 ºC. Test vessels were loosely covered to reduce evaporation. Test organisms were neither fed nor aerated during the test. All treatments were checked for mortality after 24 h and dead animals were counted.

2.6. Data analysis

The number of dead animals at each concentration was converted to percentage and the mean of each replicate was computed and compared with that of the control. Data were evaluated by one way analyses of variance (ANOVA) and multiple comparison tests by least significance difference (LSD) method. Prior to analysis, mortality percentages were arcsine-square root transformed to make percent data (percents/100) normal. A value of $p < 0.05$ was considered significant. Statistical analysis was performed using PASW Statistics for Windows (Version 18). The median lethal concentrations (LC_{50}) values and their respective confidence intervals (95%) were calculated based on the pooled data set for a given test organism by using computerized EPA probit analysis program, version-1.5 [17] as described by Finney (1971) [18]. Acute toxicity unit (ATU) and safe concentration level (SCL) were calculated using the formulas indicated by Roopadevi and Somashekar, (2012) [19]:

$ATU=100/LC_{50}\%$ (v/v).

$SCL=LC_{50}\%$ (v/v) × 0.1.

The acute toxicity unit was classified according to the acute toxicity classification system reported by Persoone *et al.,* (2003) [20].

3. Results and Discussions

The uncontrolled disposal of olive mill wastewater remains a major challenging task in olive oil producing countries because it negatively impacts the local and regional environments due to its toxicity and the lack of environmentally safe and cost-effective techniques for its treatment. Although, the Gaza Strip is one of the small olive oil producers in the region, the pollution potential of OMW generated through olive oil mill processes in the Gaza Strip however is extremely high, since each cubic meter of OMW corresponds to 100-200 cubic meters of municipal wastewaters [21].

Several works have been carried out dealing with the toxic effects of OMW on living organisms from various terrestrial and aquatic ecosystems at different taxonomical and organizational levels. In addition to its lethal effects [10, 22], OMW was known to has antimicrobial activity [11, 23], phytotixic activity [12, 24, 25], cytotoxic activity, oxidative toxicity and neurotoxic activity [14] as well as genotoxic activity [14, 26].

The results of physicochemical characterization of OMW is presented in Table (2) along with the Palestinian standards for industrial wastewater to be discharged into sewer system [27] and the recommended guidelines by the *Palestinian Standards Institute for Treated Wastewater Characteristics* to be discharged into seawater [28].

Table 2. Some physicochemical characteristics of the OMW from olive mills in the Gaza Strip compared with the Palestinian standards.

		Palestinian Standards	
Parameter	**Mean ±SD**	**Sewer system***	**Seawater****
pH	6.3 ±0.1	6– 9	6 – 9
Conductivity (mS/cm)	10.0 ±1.4	–	–
Total kjeldahl nitrogen (mg/l)	$8.63\times10^{2} \pm1.93\times10^{2}$	60	10
BOD5(mg/l)	$27.0\times10^{3} \pm4.24\times10^{3}$	500	60
COD(mg/l)	$86.0\times10^{3} \pm9.90\times10^{3}$	2000	200

SD: standard deviation.

*: Palestinian standards for industrial wastewater to be discharged into sewer system [27].

**: Recommended Guidelines by the Palestinian Standards Institute for Treated Wastewater Characteristics to be discharged into seawater [28].

The composition of OMWs mainly reflects the characteristics of the olive crops and the olive oil extraction technology employed [29]. It can be observed that, OMW is slightly acidic with high BOD, COD and nitrogen content. As compared to literature values, all measured values of each parameter tested were within the range of previously reported values for OMWs [23,30,31].

With reference to Table 2, OMW should be treated to reduce its pollution potential to acceptable levels for disposal into the public sewerage system or marine environment. At least 98.1%, 97.7% and 93% of BOD, COD and total kjeldahl nitrogen, respectively must be removed in order to meet the Palestinian standards for industrial wastewater to be discharged into the sewage systems [27]. Additionally, based on the recommended guidelines of the *Palestinian Standards Institute for Treated Wastewater Characteristics* [28], a 99.8% of both BOD and COD and 98.8 of total nitrogen must be eliminated from OMW to be safely discharged into seawater.

Mortality percentages of *B. plicatilis*, *A. salina*, *Mytilus* sp. and *B. amphitrite* exposed to the four concentration groups of OMW for 24h exposure period are presented in Figure 2.

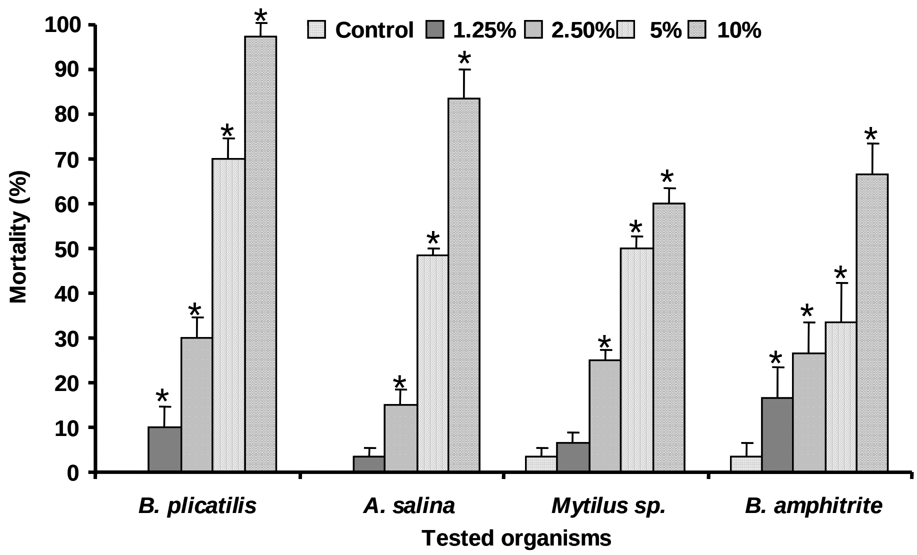

Figure 2. Average mortality percentages of *B. plicatilis*, *A. salina*, *Mytilus* sp. and *B. amphitrite* at four concentrations, 1.25, 2.5, 5.0, 10% (v/v) of OMW and control after 24h exposure period. Vertical bars represent standard errors of the mean.
* Significant difference from control value was at $p < 0.05$.

It is well known that, organism mortality due to toxicant exposure mainly depends upon its sensitivity to that toxicant, its concentration and duration exposure. *B. plicatilis* recorded the highest mortality percentage (97.5%) at the highest concentration of the OMW (10%, v/v), followed by *A. salina* (83.3%), while *Mytilus* sp. and *B. amphitrite* showed 60% and 66.7% mortality, respectively (Figure 1). The mortality percentages in exposed groups were significantly high in all tested organisms at 2.5, 5 and 10% (v/v) concentrations compared to the control group ($p < 0.05$). On the other hand, no significant difference was observed between the lowest tested concentration, 1.25% and the control except for the rotifers and barnacles (Figure 1).

It is apparent from the present study that the OMW was toxic to all test organisms and the extent of toxicity as demonstrated by the mortality percentage became progressively higher with increasing OMW concentrations. This confirms the regular mode of action, due to harmful effect of chemicals and toxicants of wastewater up to a dangerous level that caused organism death. On the other hand, the mortality in the controls of all organisms tested was ≤ 3.3%; demonstrating that the holding facilities, water and handling techniques were acceptable for the toxicity test.

The death of test organisms 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 [9]. Using a battery of well-known stress indices such as neutral red retention assay, acetylcholinesterase activity, micronucleus frequency and DNA damage, Danellakis *et al.*, (2011) [14] demonstrated the toxic effects of OMW in tissues of the mussel, *M. galloprovincialis*. Moreover, OMW was reported to have a large number of toxic chemicals such as tannins and phenolic compounds [10, 26], as well as catechol and hydroxytyrosol [32] to which the mortality of aquatic organisms could be attributed. Additionally, the mortality of aquatic organisms can be also influenced by some other factors such as COD, BOD, low molecular weight phenolic compounds, free fatty acids, as well as phenoxy radicals [33,34], pH, dissolved oxygen, solids, organic matter [32, 35, 36], temperature etc. when present beyond their maximum tolerable levels.

Acute Toxicity of Olive Mill Wastewaters from the Gaza Strip

The LC_{50} values of OMW along with their respective 95% confidence limits (lower and upper confidence limits), slope function and intercepts for *A. salina*, *B. amphitrite*, *B. plicatilis* and *Mytilus* sp. are presented in Table 3.

Table 3. LC_{50} values and 95% lower and upper confidence limits, slope and intercept (standard error) for OMW after 24 h interval for the four tested organisms, *B. plicatilis*, *A. salina*, *Mytilus* sp. and *B. amphitrite*.

		95% Confidence limits (%)		**Probit equation constants**		
Test organisms	**LC_{50} %** (v/v)	**Lower**	**Upper**	**Intercept, a (S.E.)***	**Slope, b (S.E.)***	χ^2 **
B. plicatilis	3.3	2.8	3.9	3.209 (0.263)	3.440 (0.441)	1.387
A. salina	5.1	4.4	5.9	2.744 (0.254)	3.192 (0.358)	0.238
Mytilus sp	6.5	5.1	8.9	3.401 (0.242)	1.967 (0.333)	3.132
B. amphitrite	7.1	4.7	14.7	3.610 (0.322)	1.633 (0.447)	1.565

* S.E. Standard Error
** degrees of freedom =3

The table also shows that, the calculated chi-square values were less than the tabular value (5.991) at 5% level with 3 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 3). The median lethal concentrations of OMW of the four aquatic invertebrates after 24 h exposure were found to be ranged from 3.3% to 7.1% (v/v) (Table 3). The estimated LC_{50} values were found to vary in different animal species, reflecting different tolerance limit of different species. The species *B. plicatilis* recorded the lowest LC_{50} values of 3.3% (v/v). The species *B. plicatilis* followed by the *A. salina*, *Mytilus* sp. and finally, *B. amphitrite*, with a 24 h LC_{50} values of 5.1%; 6.5 and 7.1% (v/v) respectively (Table 3). This indicates that, *B. plicatilis* was the most sensitive species while *B. amphitrite* was the least sensitive one to OMW toxicity. It is possible that 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.

Data on the acute toxicity of OMW for the four marine invertebrates tested here are scant [14,15]. Since they are considered as efficient for providing a rapid assessment of OMW toxicity, most of toxicity tests (expressed as median effective concentrations) were performed with the crustacean, *Daphnia* sp. [8,10] and the luminescent bacteria *Vibrio fischeri* [10,23,37]. Compared with the available published information about the acute toxicity of OMW to other aquatic invertebrates, however, indicated similar ranges of LC_{50} values found in the present study. For example Aggelis *et al.,* (2003) [22] reported a 24 LC_{50} value of 4.5% and 3.7% to *Artemia* sp. and the Ostracod crustacean *Heterocypris incongruens* respectively. The 24 h, LC_{50} value of OMW for the microcrustacean *Thamnocephalus platyurus* ranged from 0.73 to 12.54% [10]. Interestingly, the 24h LC_{50} value of *A. salina* estimated in the present study (5.1% v/v) was very close to that reported by Aggelis *et al.,* (2003) [22] for *Artemia* sp. (4.5 % v/v).

In the current work, the field collected invertebrates such as *B. amphitrite* and *Mytilus* sp. showed a higher tolerance to OMW than the laboratory reared invertebrates (*B. plicatilis* and *Artemia* sp.) as indicated by their high LC_{50} values (Table 3). Moreover, the 24h LC_{50} value of OMW for *Mytilus* sp. (7.1% v/v) in the present study was found to be much higher (31.5 times) than the reported 96h LC_{50} for the marine mussel, *Mytilus galloprovincialis* (0.255% v/v) collected from a mussel farm located in the Gulf of Kontinova, Galaxidi, Greece [14]. This may indirectly reveal the pollution status of the Gaza fishing port, from which mussels and barnacles were collected. Despite of the absence of scientific studies specifically addressing the environmental situation of the Gaza fishing port, taking a casual look at the site however will show many sources of pollution such as effluents from domestic sewage and discharges from shipyards. Previous studies documented that individuals from populations inhabiting polluted areas can show more tolerance to toxicant exposure than those from non-polluted areas [38] with higher LC_{50} values for the former than the latter [39].

Several studies conducted on the acute toxicity of invertebrates with different toxicants, indicated that crustacean, especially the genus *Artemia*, is the most sensitive group in short-term tests [40,41]. This is contrary to our findings where *A. salina* occupied the second order of sensitivity after *B. plicatilis*. Similar results concerning the relative sensitivity of *B. plicatilis* and *A. salina* exposed to municipal landfill leachate were reported by Tsarpali *et al.,* (2012) [42]. Indeed, a survey of the literature revealed a considerable variation in LC_{50} values not only between the different species, but also for the same species and toxicant at different conditions such as size, age and condition of test species along with experimental factors such as temperature, pH and dissolved oxygen [43]. Thus, a comparison of the LC_{50} values between different species or even in the same species at different conditions would be sometimes, impractical.

According to the acute hazard classification system reported by Persoone *et al.*, (2003) [20], acute toxicity units (ATU) greater than or equal to 10 and less than 100 ($10 \leq ATU < 100$) is considered highly toxic. In the present study, the acute toxicity units obtained for OMW were ranged from 14 to 30 (Table 4), and therefore, OMW can be considered to have a high acute toxicity to all aquatic invertebrates tested. 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 and ranged from 0.332 to 0.710% (v/v).

Table 4. Estimated acute toxicity units (ATU) and safe concentrations.

Test organism	**Acute toxic unit (ATU)**	**Safe concentration %** (v/v)
B. plicatilis	30.148	0.332
A. salina	19.643	0.509
Mytilus sp.	15.375	0.650
B. amphitrite	14.092	0.710

In conclusion, acute toxicity testing showed that OMW was highly toxic to aquatic organisms and has a potentially harmful impact on the marine environment of the Gaza Strip. Accordingly, the disposal of OMW in the Gaza Strip should be managed to minimize its negative impact to an extent that does not threaten the marine environment. In order to have effective wastewater management however, there is an urgent need to implement stringent rules and regulations to prevent or control the disposal of OMW into the environment. Further studies on toxicity of OMW and its components in the aquatic environments are required.

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