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Full Length Research Paper

Integrated system for rearing *Mugil* species in a crude oil treated seawater using a marine *Pseudomonas aeruginosa strain elnaggar1*

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This study aimed to create an integrated model system to bio-remediate escaped crude oil in marine environment and study the impact of the bioremediation processes on the aquatic living organisms using *Mugil cephalus* fries as a living case study. Three crude oil concentrations (100, 300 and 500 ppm) were tested using glass aquaria inoculated with *M. cephalus* fries. The chemical treatment was carried out using a commercial oil disperser and a microbial treatment was carried out by a marine bacterial isolate (*Pseudomonas aeruginosa* strain *elnaggar1*), the experiment was extended for 45 days. The impacts of these treatments on the growth performance and the survival percent of these *M. cephalus* fries were recorded according to the changes in their clinical signs, lengths and weights along the rearing period. The results indicated that the microbial treatment using *P. aeruginosa* is more effective for the remediation of the crude oil contaminated seawater and also for keeping the growth performance of the tested fish as similar as the untreated fries. On the other hand, the used chemical treatment led to increase the susceptibility of these tested fries towards the rot fin disease.

Key words: *Mugil cephalus,* crude oil, *Pseudomonas aeruginosa,* bioremediation, rearing.

INTRODUCTION

Petroleum production and operation produce serious ecological problems. Pollution of environment due to accidental oil spillage, seepage and rupture pipelines had been well reported (Okerentugba and Ezeronye, 2003). Moreover, oily wastewater, especially from oil field, has posed a great hazard for terrestrial and marine eco-systems. The traditional treatments of oily wastewater, such as containment and collection using floating booms, adsorption by natural or synthetic materials, etc., cannot degrade the crude oil thoroughly. So far, the biodegradation processes suggest an effective method where the crude oil considered as a carbon source for microbial growth, which results in the breakdown of the oil to lower molecular weight compounds (Guo-liang et al., 2005).

However, on creation a system include three major components oil, fish and bacteria many challenges appeared, firstly, Giles et al. (2006) determine that the exposure of the mullet (*Mugil cephalus*) to crude oils had relationship with the fin rot disease. In addition, rearing of *M. cephalus* in presence of some bacterial strains such as *Vibrio anguillarum* and *Aeromonas hydrophila* led to a fish disease known as 'red spot' (Burke and Rodgers, 2006; Rodgers and Burke, 2006). Moreover, the bacterial utilization of the crude oil components requires complex cell surface adaptation to allow adherence to oil and precede the degradation process. Meanwhile, it was mentioned that the use of probiotics for disease control in aquaculture is an area of increasing interest. Probiotics have been defined by the World Health Organization's-Food and Agriculture Organization (FAO, 2001) as ''live

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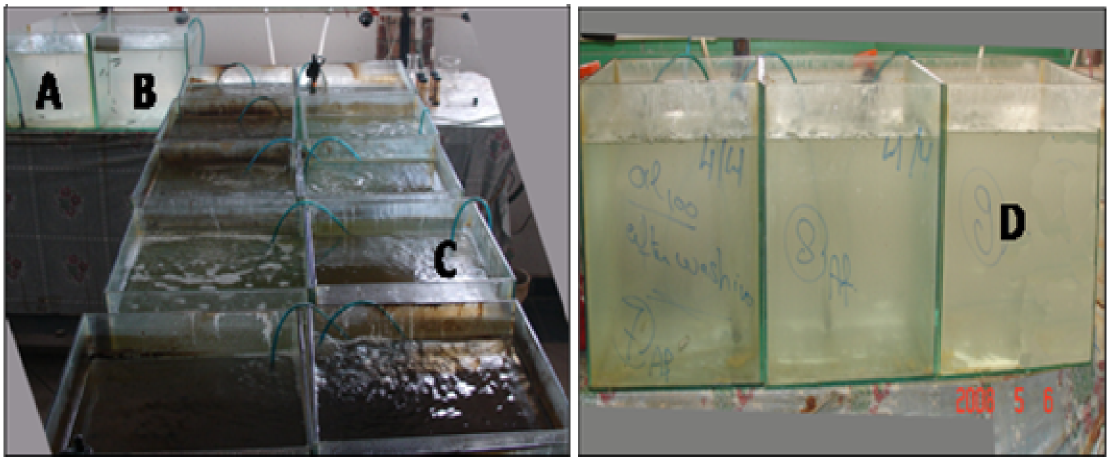

Figure 1. Photographs shows experimental frame work and the used glass aquaria filled with untreated seawater (control) (A); the *P. aeruginosa* in contact with *M. cephalus* fries -blank (B); the oily contaminated seawater under treatments (C) and; the glass aquaria of the washing process (D).The feeding regime.

microorganisms which when administered in adequate amounts confer a health benefit on the host." In the last decade, several gram-negative and gram-positive bacteria have been evaluated *in -vitro* or *-vivo* for their potential to inhibit fish-pathogenic organisms and overcome infections in fish and larvae in aquaculture (Irianto and Austin, 2002). On the other hand, many authors used *Pseudomonas* sp. for the decontamination of wastes and such biodegradation processes proved to result in little or no impact on environment (Cernigilia, 1992; Obayori et al., 2009).

Thus, the goal of this study is to create an integrated system using a marine local bacterial isolate *Pseudomonas aeruginosa strain elnaggar1,* which isolated from Sidi Kerir, West Alexandria, to enable the rearing of *M. cephalus* fries in presence of crude oil contaminated seawater. In addition, to evaluate the use of this marine bacterial isolate as a probiotic agent for controlling the rot fin disease which attack the *M. cephalus* when exposed to the crude oils.

MATERIALS AND METHODS

Fish stock

Experiments were carried out at National Institute of Oceanography and Fisheries, Alexandria, Egypt. M. cephalus post larvae were captured using fishing nets (1 mm mesh) from the eastern harbor of Alexandria. They were transported and acclimated in two 100 L-glass tanks filled with seawater from the collecting site (33 salinity and 20ºC temperature). The tanks were equipped with constant aeration and kept under natural photoperiod (13 L: 11 D). The post larvae remained in the tanks for two weeks, according to (Sampaio et al., 1998).

Before the post larvae were distributed in the experimental aquaria, 50 individuals were randomly selected for the detection of the initial weight and length. Then the post larvae were transferred to ten 30 L glass aquaria (30 × 30 × 50 cm) filled with 27 L seawater at densities 10 mullet/aquarium.

Experimental design

The impact of three crude oil concentrations 100, 300 and 500 ppm was determined in these aquaria $O\&F_{100}$, $O\&F_{300}$ and $O\&F_{500}$ respectively. The impact of 10% inoculums of the marine bacterial strain *P. aeruginosa* was estimated in an aquarium (B&F). Moreover, the bacterial treatments of 100, 300 and 500 ppm crude oil concentrations were carried out in these aquaria $B\&F\&O_{100}$, $B\&F\&O_{300}$ and $B\&F\&O_{500}$, respectively, using 10% inoculums of the marine bacterial strain *P. aeruginosa* strain elnaggar1.

In addition, the chemical treatments of 300 and 500 ppm crude oil were carried out in the aquaria $chem._{300}$ and $chem._{500}$, respectively, using a commercial oil disperser. A control was made using untreated seawater without any additives (C) as shown in Figure 1. The experimental period was extended for 45 days; it was divided into 15 days for adaption and acclimatization, 15 days for crude oil treatments and 15 days for washing process.

The feeding regime was applied at 5% body weight per day throughout the experiment; the frequency of feeding was maintained as twice a day for six days a week. The artificial diet was analyzed for moisture, crude protein, ether extract and ash according to standard AOAC methods (AOAC, 1980) (Table 1).

Microbiological examinations

Bacterial strain

P. aeruginosa was isolated from Sidi Kerir bottom water sample through enrichment process on crude oil. The isolate has been described as a gram-negative, rod-shaped and aerobic bacterium. The partial identification process was carried out according to Bergey's manual, section-4 "gram negative aerobic rods and cocci" (Sneath et al., 1986).

Molecular identification process

This process was carried out at Mubarak City for Scientific Re-search and Technology Applications, Arid Land Institute, Molecular

Table 1. The composition of the artificial diet used for *M. cephalus* feeding regime.

Ingredients	Percentage
Fish meal	25.00
Soybean meal	12.00
Broken corn	17.00
Wheat milling by-product	45.00
Mineral mixture	0.70
Vitamin premix	0.30
Chemical analysis	
Dry matter	87.12
Crude protein	32.1
Either extract	2.63
Crude fiber	2.46
Ash	11.61
Nitrogen free extract	51.1

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DNA extraction

DNA was extracted from overnight pure culture of this marine isolate using Qiagen DNeasy kit (QIAGEN-Inc., Germany) and Genomic DNA purification kit (Promegal). The procedure was identical to that recommended by the manual instructions. The preparations were analyzed on a 0.7% agarose gel and then determined spectrophotomertically (Sambrook et al., 1989).

PCR-amplification and sequencing of 16S rDNA gene.

The amplification of the genomic DNA was carried out by a PCR using *Pseudomonas* 16S rDNA gene primer PA-SS-F' GGGGGATCTTCGGACCTCA, PA-SS-R' TCCTTAGAGTGCCCAC CCG, according to Spilker et al. (2004).

Nucleotide sequence accession number

The GenBank accession number for the 16S rDNA sequences generated in this study is GQ505252, the isolate was identified as *P. aeruginosa* strain *elnaggar1*.

Culture conditions

The culture medium used for isolation and activation of P. aeruginosa was modified from that of Guo-liang et al. (2005), where the seawater was used instead of distilled water. The composition of this basal mineral salt medium was as follow (g/l): $NaNO_3$ 4.0, NaCl 1.0, KCl 1.0, $CaCl_2{\cdot}2H_2O$ 0.1, KH_2PO_4 3.0, $Na_2HPO_4{\cdot}12H_2O$ 3.0, $MgSO_4$ 0.2, $FeSO_4{\cdot}7H_2O$ 0.001; 2 ml trace element stock solution composed of (g/L): $FeCl_3{\cdot}6H_2O$ 0.08, $ZnSO_4{\cdot}7H_2O$ 0.75, $CoCl_2{\cdot}6H_2O$ 0.08, $CuSO_4{\cdot}5H_2O$ 0.075, $MnSO_4{\cdot}H_2O$ 0.75, H_3BO_3 0.15, $Na_2MoO_4{\cdot}2H_2O$ 0.05. The initial pH was adjusted to 7.7 and the incubation was done for 24 – 48 h at 25ºC.

Sampling processes and microbial examination processes in the oily contaminated seawater

Water samples for microbiological analyses were taken regularly from the rearing fish aquaria after zero, 2, 3, 4, 7 and 14 days of the treatment and in addition, after one week of the washing process. 1 ml of these seawater samples was used aseptically to inoculate 9 ml of sterilized culture medium and then incubated for 24 – 48 h at 25ºC. Then, the dry weight of the bacterial growth was estimated in mg/100 ml according to Dalgaard et al. (1994).

Examination of skin

The skin of three treated fish per aquarium were swapped and re-suspended in 5 ml sterile phosphate-buffered saline (PBS), it composed of; 8.0 g of NaCl, 0.3 g of KCl, 0.73 g of NaH_2PO_4 and 0.2 g of K_2HPO_4 to 1 liter of de-ionized water, pH 7.4. All samples were 10-fold diluted, squeezed by hand for few minutes and then spread onto nutrient agar plates which composed of (g/l): 3; beef extract, 5; peptone and 20; agar using a glass spreader (5 cm). The total bacterial count, colony forming unit (cfu/ml) was estimated in each sample according to Buller (2004). Sterile gloves, bags, swabs, and glass beakers were used for sampling.

Examination of muscle

The head, internal organs and the tail of three examined fish per aquarium were removed under sterile conditions. Then 1 g of the muscle part was transferred to sterile tubes contain 1 ml PBS. All samples were 10-fold diluted, squeezed by hand for a few minutes and spread onto nutrient agar plates as mentioned above. The total bacterial count, colony forming unit (cfu/ml) was estimated in each sample according to Buller (2004).

Examination of internal organ

The internal organs of each examined fish were removed under sterile condition using sterile forceps and transferred to sterile tubes contain 1 ml PBS. All samples were 10-fold diluted, squeezed by hand for a few minutes and spread onto nutrient agar plates s mentioned above. The total bacterial count, colony forming unit (cfu/ml) was estimated in each sample according to Buller (2004).

Chemical treatment

The chemical treatment of the used crude oil was carried out using 100 ppm of a commercial OIL DISPERSER. The physical properties of this disperser were estimated as follows: Clear pale yellow liquid, Sp; Gravity at 25°C = 1.02 - 1.025 g/cm^3; Solubility, soluble in water; Flash point, no; Flammable limits in air, no and; the pH for 1% aqueous solution = 8.25.

Determination of crude oil using gas chromatography extraction

1000 ml of each collected water samples were extracted three times with 100 ml of dichloromethane in a separating funnel. Sample extracts were combined and concentrated to 5 ml using a rotary evaporator under reduced pressure. Finally, samples were concentrated under a gentle stream of pure nitrogen to a volume of 1 ml (UNEP/ IOC/ IAEA, 1992).

Clean-up and fractionation process

Clean-up and fractionation was performed prior to gas chromatograph/flame ionization detector (GC/FID). The extracted volume was passed through the silica column prepared by slurry packing with 10 g of silica, followed by 10 g of alumina and finally 1 g of anhydrous sodium sulphate. The aliphatic fraction (F1) was sequentially eluted from the column using 25 ml of hexane. However, the unsaturated aromatic fraction (F2) was eluted with 60 ml of hexane and dichloromethane (80:20; V/V). Both of F1 and F2 fractions were concentrated using a gentle stream of pure nitrogen to about 0.2 ml, before being injected into GC/FID (UNEP/ IOC/ IAEA, 1992).

Gas chromatography

All samples were analyzed by a Hewlett Packard 5890 series II GC gas chromatograph equipped with a flame ionization detector (FID). The instrument was operated in split less mode (3 µl split less injection) with the injection port maintained at 290°C and the detector maintained at 300°C. Samples were analyzed on a fused silica capillary column HP-1; 100% dimethyl polysiloxane (30 m length, 0.32 mm i.d, 0.17 µm film thickness). The temperature was programmed from 60 - 290°C, changing at a rate of 3°C /min and maintained at 290°C for 25 min. The carrier gas was nitrogen flowing at 1.2 ml/ min (UNEP/ IOC/ IAEA, 1992).

Quantification and mixture preparation

Aliphatic standard mixture (100 µg/ml) brought from MERCK was used for F1 analysis, this standard mixture containing C11,C12, C13, C15, C17, pristane, C18, phytane, C19, C20, C21, C22, C23, C24 and C30. Chlorofluorobenzene (CFB); 20 µg/ml was used as internal standard for aliphatic fraction. In addition, a stock solution containing the following PAHs was used for quantification of hydrocarbons: naphthalene, acenaphthylene, acenaphthene, fluorene, phenathrene, anthracene, fluoranthene, benzo(a) anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, pyrene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(ghi) perylene and indeno(1,2,3-cd) pyrene by dilution to create a series of calibration standards of PAHs at 0.1, 0.25, 0.5, 0.75, 1.0, 2.0, 5.0 and 10 µg/ml. The detection limit was ≈ 0.01 µg/ml for each PAH. For analytical reliability and recovery efficiency of the results, 6 analyses were conducted on PAH reference materials, HS-5 and 2974 (provided by EIMP-IAEA). The laboratory results showed recovery efficiency ranging from 92 - 111% with coefficient of variation (cv) 8 - 14% for all studied pollutants (16 PAHs fractions). All solvents were pesticide grade purchased from Merck and appropriate blanks (1000 fold concentrates) were analyzed.

Statistics

The statistical analyses of the data were carried out in triplicates using ANOVA test and the least significant difference L.S.D. according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

The isolation of the used bacterial strain was carried out from a bottom water sample collected from Sidi Kerir, West Alexandria, where many industrial oil companies pour their wastes. The selection process was based on its high ability to degrade crude oil through enriched water samples with oil. The identification process was carried out at Mubarak City for Scientific Research and Technology Applications using the molecular technique. The results indicated the isolation of a new bacterial strain identified as *P. aeruginosa strain elnaggar1* with an associated number of GQ505252 in the data base of the GenBank library.

However, the growth performance and the survival percentage of *M. cephalus* were estimated in the examined oily contaminated seawater after 30 and 45 days of the rearing process (Table 2). The obtained data showed in general that the bacterial treatment led to slight impacts on the growth performance of the *M. cephalus* compared to the untreated fish (control), while the chemical treatment showed serious impacts on both the growth performance and the survival percentage of *M. cephalus* especially on testing the 500 ppm oil degradation process ($Chem_{500}$) compared to the control.

Moreover, it was observed that after 30 days of the rearing period the addition of 100, 300 and 500 ppm crude oil in the tested aquaria $O\&F_{100}$, $O\&F_{300}$ and $O\&F_{500}$, respectively, led to severe effects on the growth rates of *M. cephalus* fries. The daily weight gain was reduced by 71.5, 78.6 and 78.6% compared to the untreated fish (control), respectively and the daily length gain was reduced by 46.7, 46.7 and 53.3%, respectively. While, the chemical treatment of 300 ppm oil ($Chem_{300}$) led to a reduction of 78.6 and 46.7% in the daily weight gain and the daily length gain, respectively, compared to the control. Moreover, the chemical treatment of 500 ppm crude oil in the aquarium $Chem_{500}$ led to a complete death of the tested *M. cephalus* fries within two days of the treatment.

On the other hand, the *P. aeruginosa strain elnaggar1* in contact with these fish fries (F&B) let to 10.7 and 6.7%, reduction percentage in the daily weight gain and the daily length gain respectively, compared to the control. In addition, the microbial treatment which carried out in the aquaria $O\&F\&B_{100}$, $O\&F\&B_{300}$ and $O\&F\&B_{500}$, it led to a reduction of 7.1, 10.7 and 39.3% in the daily weight gain, respectively and a reduction of 6.7, 20 and 33.3% in the daily length gain compared to the control.

However, after 45 days of the rearing process these estimated parameters showed to be highly affected by both the presence of the crude oil and the use of the oil disperser in the treatment process. The results showed that the presence of the crude oil in concentrations ranged form 100 to 500 ppm led to a reduction in the daily weight gain and the daily length gain of the *M. cephalus* fries, it ranged from 4.4 mg/fish/day to 7.8 mg/fish/day and from 0.24 mm/fish/day to 0.39 mm/fish/day, respectively compared to the untreated fish (45.7 mg/fish/day and 0.94 mm/fish/day) While the use of the oil disperser led to a daily weight gain of 12.89 mg/fish/day and a daily length gain of 0.56 mm/fish/day. Moreover, the data presented in Tables 2 and 3 showed

Table 2. The effect of the microbial and chemical crude oil treatments on the growth performance, survival percentage and feed utilization parameters of *M. cephalus* after 0, 30, 45 days of rearing.

Estimated parameters	Treatments									
	Control	B&F	O&F$_{100}$	O&F$_{300}$	O&F$_{500}$	O&B&F$_{100}$	O&B&F$_{300}$	O&B&F$_{500}$	Chem$_{300}$	Chem$_{500}$
At zero time (Stocking data)										
Av. Initial weight (g) ± SD	0.04 ± 0.21	0.04 ± 0.21	0.04 ± 0.21	0.04 ± 0.21	0.04 ± 0.21	0.04 ± 0.21	0.04 ± 0.21	0.04 ± 0.21	0.04 ± 0.21	0.04 ± 0.21
Av. Initial length (cm) ± SD	1.1 ± 0.32	1.1 ± 0.32	1.1 ± 0.32	1.1 ± 0.32	1.1 ± 0.32	1.1 ± 0.32	1.1 ± 0.32	1.1 ± 0.32	1.1 ± 0.32	1.1 ± 0.32
After 30 days of rearing										
Av. final weight (g)	0.32	0.29	0.1	0.1	0.12	0.3	0.29	0.21	0.1	Death
Av. final length (cm)	2.6	2.5	1.9	1.8	1.8	2.5	2.3	2.1	1.9	
Gain in weight (g/fish)	0.28	0.25	0.08	0.06	0.06	0.26	0.25	0.17	0.06	
Daily weight gain (mg/fish/day)	18.67	16.67	5.33	4	4	17.33	16.67	11.33	4	
Daily length gain (mm/fish/day)	1.5	1.4	0.8	0.8	0.7	1.4	1.2	1	0.8	
After 45 days of rearing										
Av. final weight (g) ± SD	2.1 ± 0.04	1.86 ± 0.01	0.39 ± 0.02	0.28 ± 0.07	0.24 ± 0.01	1.75 ± 0.28	2.00 ± 0.66	1.62 ± 0.23	0.62 ± 0.86	
Av. final length (cm) ± SD	5.35 ± 0.67	4.25 ± 0.33	2.85 ± 0.22	2.5 ± 0.38	2.2 ± 0.11	5.1 ± 0.87	5.1 ± 0.33	4.7 ± 0.12	3.6 ± 0.19	
Gain in weight (g/fish)	2.06	1.77	0.35	0.24	0.2	1.71	1.96	1.58	0.58	
Daily weight gain (mg/fish/day)	45.78	39.33	7.78	5.33	4.44	38	43.56	35.11	12.89	
Daily length gain (mm/fish/day)	0.94	0.7	0.39	0.31	0.24	0.89	0.89	0.8	0.56	
Instant daily growth (IDG)	3.71	3.59	2.85	2.78	2.76	3.56	3.67	3.5	2.99	
Survival rate (%)	90	80	50	40	30	80	70	70	60	
Feed utilization data										
Feed conversion ratio (FCR)	2.16	2.23	2.98	3.1	3.76	3.41	3.53	3.83	3.71	
Protein efficiency ratio (PER)	2.96	1.99	1.65	1.41	1.23	1.33	1.06	1.06	1.01	

also the impact of the bacterial treatment (*P. aeruginosa*) on the growth performance and the survival rate of *M. cephalus* after 45 days of rearing, which including two weeks for adaptation, two weeks for treatment and two weeks for washing. It was observed that the microbial treatment of 100, 300 and 500 ppm crude oil had the slight impacts on the rearing process of *M. cephalus* fries compared to the untreated fish. The daily weight gain and the daily length gain were fish/day and from 0.8 to 0.89 mm/fish/day, ranged from 35.11 to 43.56 mg/fish/day and from 0.8 to 0.89 mm/fish/day, respectively. While the presence of *P. aeruginosa* in the aquaria F&B, O&F&B$_{100}$, O&F&B$_{300}$, and O&F&B$_{500}$ led to a survival percentage ranged from 70 to 80% compared to the control (90%). On the other hand, the survival percentage was reduced to 50, 40 and 30% on the addition of the crude oil in the tested aquaria O&F$_{100}$, O&F$_{300}$ and O&F$_{500}$, respectively.

However, the data presented in Figure 2 showed that with increasing of the oil concentration from 100 to 300 to 500 ppm in these tested aquaria the maximum bacterial dry weight of *P. aeruginosa* was increased from 130 mg/l to 240 mg/l to 900 mg/l, respectively, and it was obtained mainly after the 4th day of treatment. In addition, the presence of the crude oil and the oil disperser in contact with these fish fries led to inhibit the bacterial growth in the surrounding media during the first three days of the treatment. Moreover, the washing process showed the insignificant impact of this integrated system on the surrounding media

Table 3. The statistical analysis of the growth parameters of *M. cephalus* along 45 days of the rearing process under microbial and chemical treatments.

Estimated Parameters	Treatment									
	Control	B&F	O&F$_{100}$	O&F$_{300}$	O&F$_{500}$	O&B&F$_{100}$	O&B&F$_{300}$	O&B&F$_{500}$	Chem$_{300}$	LSD
Daily weight gain (mg/ fish/day)	32.2^{a*}	28.05^{c}	6.56^{f}	4.67^{g}	4.21^{g}	27.67^{c}	30.08^{b}	23.24^{d}	8.45^{e}	0.263
Daily length gain (mg/fish/day)	1.27^{a}	1.05^{b}	0.59^{de}	0.55^{ef}	0.47^{f}	1.2^{a}	1.05^{b}	0.9^{c}	0.69^{d}	0.118

*Mean values in the same row which have the same letters are insignificantly.

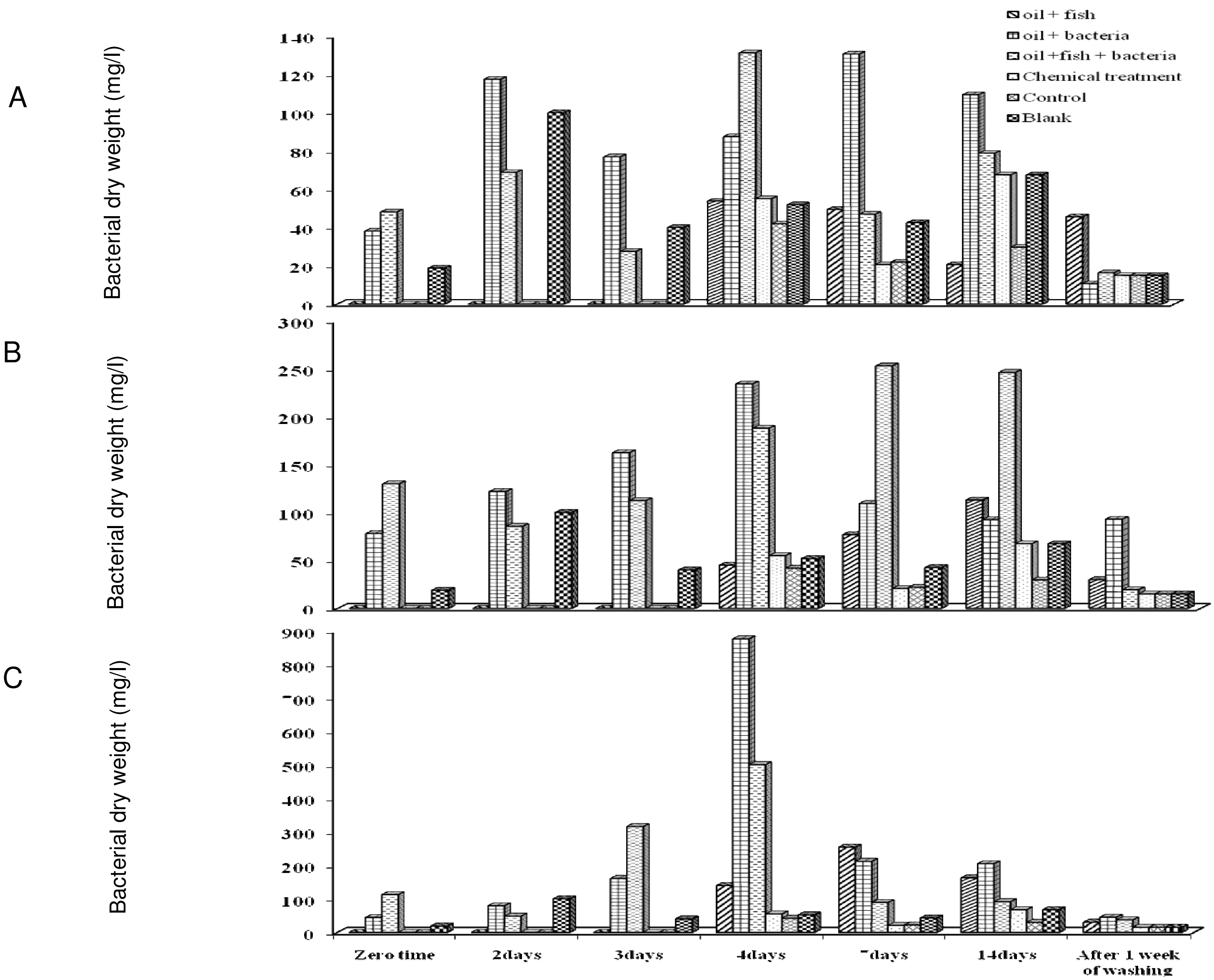

Figure 2. The bacterial dry weight in the surrounding media of the contaminated aquaria (O&F, B&F, O&F&B, Chem., F&B and the control) during 15 days of 100ppm (A), 300 ppm (B) and 500 ppm (C) crude oil treatment and after one week of washing process.

on the surrounding media since after a week of washing, it led to reduce the bacterial content to a level slightly similar to that of the control.

Moreover, the statistical analysis presented in Table 4 showed that with increasing oil concentration the efficiency of the microbial treatment increase. The maximum mean degradation rate was observed after four treatment was O&F&B$_{300}$ followed by O&F&B$_{500}$ and days

Table 4. The remediation rates of the soluble crude oil fractions by microbial and chemical treatments.

Time (day)	Remediation rate (ppm/day)					
	Microbial treatment			Chemical treatment		Mean*
	$O\&F\&B_{100}$	$O\&F\&B_{300}$	$O\&F\&B_{500}$	$Chem_{300}$	$Chem_{500}$	
1	1.78	12.5	7.9	3.2	9.56	6.99[b]
4	5.3	27	36.3	6.3	20.8	19.14[a]
7	3.4	3.6	19.7	5.6	4.9	7.44[b]
14	2.6	0.36	6.2	0.057	0.36	1.92[c]
Mean*	3.27[c]	10.86[b]	17.53[a]	3.79[c]	8.91[b]	

*Mean values in the same column or in the same row which have the same letter are insignificantly different at P < 0.01 while the mean values with different letters are significantly different at P < 0.01.

Table 5. The microbiological examinations of *M. cephalus* after 45 days of rearing.

Treatments	Bacterial count CFU × 10^2/100 ml			
	Muscle	Skin	internal organs	Mean*
Control	2	0.8	2	1.6[d]
B&F	1.3	3.8	3.8	2.97[c]
$O\&F\&B_{100}$	2.2	3.6	2.3	2.7[c]
$O\&F\&B_{300}$	2.5	6	4	4.16[b]
$O\&F\&B_{500}$	1.5	4.7	5	3.73[b]
$Chem_{300}$	4.8	10	8.8	7.87[a]
Mean*	2.38[c]	4.81[a]	4.31[b]	

Mean values in the same column or in the same row which have the same letter are insignificantly different at P < 0.01 while the mean values with different letters are significantly different at P < 0.01.

of the experiment regard less to the used treatment (19.14 ppm/day) and the most effective microbial $O\&F\&B_{100}$ using the marine *P. aeruginosa*, the degradation percent were 36, 29 and 21.2%, respectively, compared to the original crude oil added to these aquaria.

On the other hand, the chemical treatment of 300 and 500 ppm crude oil using a commercial disperser led to degradation percentages of 8.4 and 16.6%, respectively, compared to the original crude oil added to these aquaria. This great effectiveness explained by several authors they mentioned this bacterial species able to produce the bio-surfactant of rhamnolipid in the presence of the crude oil which facilitate the biodegradation process (Balba et al., 2002; Tang et al., 2007; Urum et al., 2003). Moreover, the production of this biosurfactant in the surrounding medium of the bacterial cells showed to extract the lipopolysaccharides from the cellular envelope which increase the cell hydrophobicity and it subsequently stimulates the uptake process of the hydrocarbon droplets via the direct contact with these bacterial cells (Guo-liang et al., 2005).

Moreover similar results were obtained by Norman et al. (2002), they studied the effect of extracting the lipopolysaccharides (LPS) from the cell surfaces of *P. aeruginosa* strains on the crude oil degradation (n-alkane). It was found that the cell hydrophobicity increased and the degradation of n-alkane increased. On the other hand, Guo-liang et al. (2005) observed that neither cell growth nor rhamnolipid production were obtained when *P. aeruginosa* grown on crude oil as a sole carbon source.

However, the impact of the microbial and the chemical treatments on the total bacterial count of the muscle, skin and the internal organs of the tested *M. cephalus* was presented in Table 5. It was observed that the most affected fish part was the skin followed by the internal organs. The lowest bacterial count was estimated in the fish muscle. The chemical treatment led to increase the fish susceptibility towards the bacterial accumulation compared to the control. It showed a high significant difference at P < 0.01 in accumulating the bacterial counts in these examined fish the mean bacterial count was 7.87 × 10^2 cfu/100 ml compared to that of the control(1.6 × 10^2 cfu/100 ml). However, the bacterial treatments showed lower bacterial accumulation in these examined fish parts especially in case of $O\&F\&B_{100}$ and the O&B the estimated counts were 2.7 and 2.97 × 10^2 cfu/100 ml, respectively.

Moreover, the impacts of these all treatments on the morphological symptoms of the tested fish were summarized in Figure (3). The data showed a significant susceptibility of *M. cephalus* to the rot fin disease especially the caudal fin it almost degraded due to the

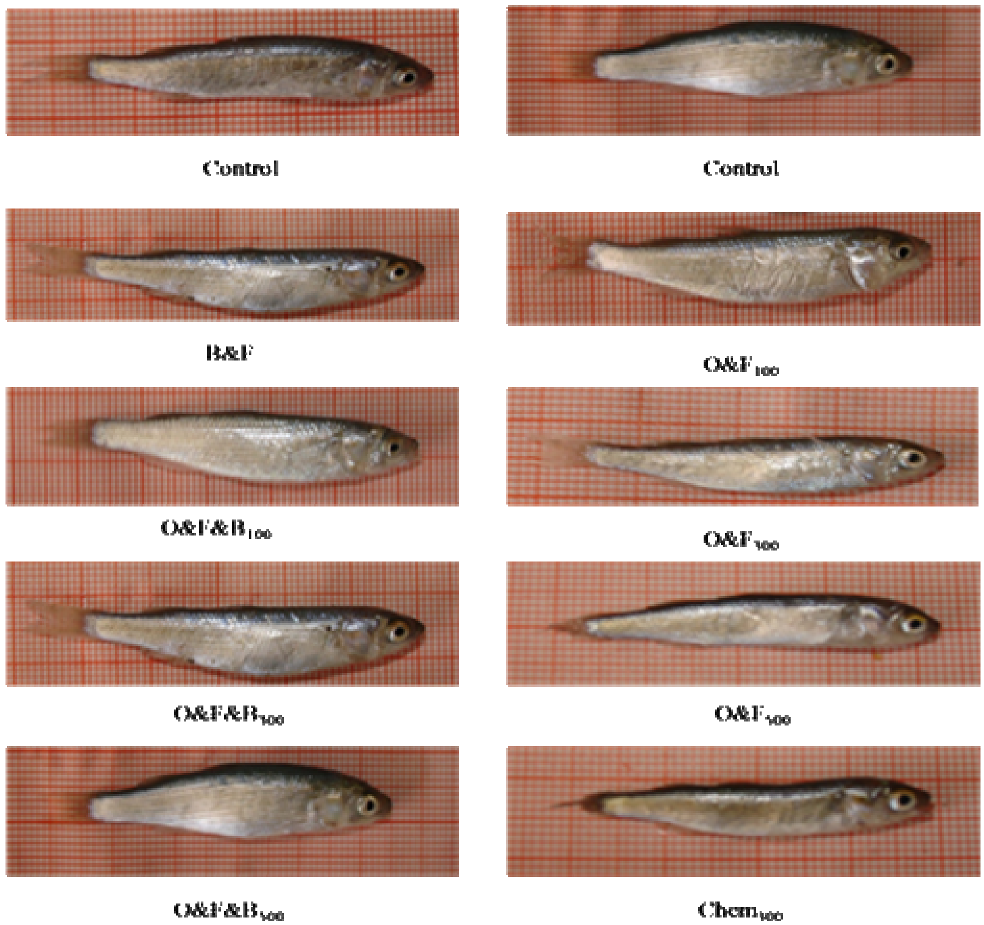

Figure 3. Photographs show the effects of microbial and chemical treatments on the growth performance of *M. cephalus* after 45 days of rearing.

presence of either high crude oil concentration or even the presence of the oil disperser in the surrounding media. Similarly, Douglas and Yarbrough (2006) showed that after Six to eight days of the oil exposure all the examined mullet which taken from the treated ponds had varying degrees of fin rot on one or more of their fins. Where, the fin erosion involved primarily the caudal, pectoral and pelvic fins, the caudal fin was the most severely damaged. In addition, they observed the degree of damage was varied from a slight discoloration with no visible fraying to complete erosion of all of the fin elements. They isolated a gram negative rod tentatively identified as *Vibrio* sp. and they considered it as the primary pathogen responsible for the fin erosion. Mean while, it can be noticed this microbial treatment may kept the survival percent and the growth performance of the tested fish as partially similar as that of the control and this can be explained by Spanggaard et al. (2001), they showed the fish pathogenic bacterium *V. anguillarum* growth was most prominently inhibited by *Pseudomonas* spp. using the well diffusion assay and the all disease-protecting strains were pseudomonads. Moreover, they mentioned pseudomonads are typically siderophore producers and the addition of live bacterial cultures to fish-rearing water may thus improve survival of the fish. However, these results were also in agreement with that of Gram et al. (1999), they observed the combined pro-biotic treatment using *Pseudomonas fluorescens* resulted in a 46% reduction of calculated accumulated mortality; it was 25% after 7 days in the probiotic-treated fish, wherea s mortality was 47% in fish not treated with the probiont.

Moreover, although the exposure of the *M. cephalus* to the water-soluble fractions (WSF) of crude oil increase the susceptibility to bacterial infection it was noticed that the muscle of the examined fish was the lowest infected part. This can be explained by the work done by Thomas (2006); he studied the effect of the exposure of the mullet to the water-soluble fractions (WSF) of crude oil and two fuel oils. It was obtained that these tested oils altered the ascorbic acid (AsA) content of several striped mullet, *M. cephalus* tissue. In addition, the exposure to sub-lethal concentrations of the WSFs caused a depletion of AsA reserves in brain, gill, kidney and liver tissues, but not in muscles.

Conclusion

The results indicated the use of the chemical treatment for escaped crude oil in marine environment it may led to increase fish susceptibility towards bacterial accumulation especially on the skin part compared to the untreated fish. Moreover, the bacterial treatment using the marine *P. aeruginosa* led to the degradation of the oil with low impact on the fish in the surrounding medium which more or less seemed to be similar to the untreated fish. So, it could concluded it may be useful to use such integrated microbial system for crude oil bioremediation in marine contaminated areas and in the same time it could act as a potent probiotic agent for the reared *M. cephalus* which enable them to control the fish diseases like rot fins and tails.

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