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**HISTOPATHOLOGICAL EFFECTS OF THE 4-NONYLPHENOL ON THE GILL,  
LIVER AND KIDNEY IN THE EMBRYO OF THE IRANIAN STURGEON  
(*ACIPENSER PERSICUS*)**

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**ABSTRACT**

Nonylphenols have comprehensive usage in plastic, cleaning products, resins and stabilizers in industry. In relation to adverse effects of the nonylphenols on the aquatic animals a few works were done in the Caspian Sea . The present study demonstrated the effects of the 4-nonylphenol on the gill, liver and kidney in the embryo of the Iranian sturgeon (*Acipenser persicus*) as biomarkers . 270 pre-larvae of the Iranian sturgeon in the 3 treatments and 3 replications ( two sub-lethal concentrations 40 and 70  $\mu\text{g/l}$  and control ) was applied. The experiment was carried out semi-static. For light microscopic studies, dead and deformities embryo in the three phases (10 , 20 and 30 days post hatching ) were fixed by immersion in Bouin fluid for 24 hours and then samples transferred to ethanol 70%. Then process of providing tissue's prelarvae was done. The histopathological alterations were found in the gill , hyperplasia, severe lesions tissue and rupture of lamellar epithelial . The main alterations in the liver were vacuolated hepatocytes, central necrosis and cell degeneration. The most alterations were found in the kidney , hypertrophied nucleus cell of tubules and degeneration of the bowman's capsule. The most alterations and the

maximum mortality of the prelarvae's *Acipenser persicus* was observed in the tertiary developmental period and 70 µg/l concentration. The sensitivity of the embryo's Iranian sturgeon in relation to 4-nonylphenol increased during development.

**Keywords:** Nonylphenol, *Acipenser persicus*, tissue necrosis, hypertrophied nucleus, hepatocyte

## INTRODUCTION

The crude oil, organic acids, heavy metals and other pollutants are the hazardous components of the marine ecosystems. The pollution of the Caspian Sea are, first of all, oil, petroleum products and phenols (Chicherina *et al.*, 2004). Nonylphenol (C<sub>15</sub>H<sub>24</sub>O) is a petroleum product (King, 2005) and a derivation of the phenol compounds that are charged into the environment from sewage treatment plants via secondary effluents and about 90% of nonylphenol via sewage slugs to form the nonylphenol ethoxilates (Ahel *et al.*, 1994). Nonylphenol have comprehensive usage in plastic and cleaning products (Jamshidi *et al.*, 2012) and it is used in production of resins and stabilizers in industries (ECB, 2002)<sup>1</sup> and has been earlier also used as softener in PVC-plastic in food packing materials (Rosqvist *et al.*, 2003). In spite of natural factors that form the conditions of life activities of hydrobionts and the state of the marine ecosystems, the economic activity in the

Caspian Sea has been increased since the second half of the 20th century; it included the damming of the Volga, greater water consumption for industrial and agricultural use, excessive use of chemical fertilizers in agriculture, larger volumes and rates of development of marine biological resources and mineral resources of the shelf. Such growth of anthropogenic load on the marine ecosystems led to essential changes in the state of the abiotic and biotic component of them (Chicherina *et al.*, 2004), again, a sharp decrease in the diversity of the benthic fauna of the Caspian Sea has been reported. In the northern part, the diversity has decreased from 78 to 46 species, and in the southern and central parts, the number of species has decreased to one third. Bulk stocks of commercial fish species have significantly reduced in the last decades; Especially, the sturgeon population has suffered. Twenty years ago, about 20 - 25,000 tons of sturgeons were harvested in the Caspian Sea annually. Over the last 20 years, the total catch has decreased to 90% (Jafari 2010). Alkylphenols and alkylphenol

<sup>1</sup> European Commission Joint Research Centre, 2002

ethoxilates have been used since 1950s in Europe and United State of America (O.E.C.D. , 2000) but usage of them decreased gradually until 2000s (Öberg et al.,2005) . In Iran , a few studies about Alkylphenols were done ; For example in 2012 , Mortazavi and his colleagues detected concentrations of 4-nonylphenol , octhylphenol and bisphenol A in Anzali wetland . The maximum concentrations of these alkylphenols were 29 , 4.3 and 7  $\mu\text{g/g}$  dry weight , respectively . In relation to adverse effects of the phenol compounds on the aquatic animals a lot of work was done . For example , according to a research done by kammann and his colleagues (2009) on the acute toxicity of 353-nonylphenol and its metabolites for zebrafish embryos , this alkylphenol compound and its metabolites leads to lethal and non-lethal malformation ( coagulation , missing heart beat and missing tail detachment ) in zebrafish embryos . In the other study, the effects of 4-nonylphenol on sex differentiation and puberty in mosquitofish (*gambusia holbrooki*) by Dreze et al. (2000) was investigated . According to this research , in group exposed to 0.5 and 5.0 mg/l , sex-ratio did not differ from unity but incompletely developed gonopodium was

observed in several individuals . Individuals exposed to 50 mg/l 4-nonylphenol exhibited female or undeveloped gonads , while gonadal sex-ratio did not statistically differ from unity in control group . Percentage of undeveloped gonads increased with 4-nonylphenol concentration . Additional observations demonstrated hepatic histopathology in fish exposed to the highest concentration and growth reduction dependent on 4-nonylphenol concentration . In the sturgeon fish such as the Iranian sturgeon (*Acipenser persicus*) the period of reproduction is 8 to 15 years in males and 12 to 18 years in females (Chebanov et al. , 2011) . The decrease of depth of the lake and rivers made the study of effects of pollutions on them very difficult . So , in this study , the embryos of the Iranian sturgeon was exposed to sub-lethal concentrations 4-nonylphenol at the three periods in the Iranian sturgeon and then histopathological effects of the 4-nonylphenol on the gill , liver and kidney as biomarkers in the embryos were studied.

#### MATERIAL AND METHODS

Healthy *Acipenser persicus* prelarvae on the embryonic 38 phase (3dph) were collected randomly from Shahid Marjani Sturgeon Propagation and Cultivation Centre , Golestan province , Iran on

March 19, 2014. 270 pre-larvae were placed into 9 plastic pails to 3 liter volumes, with 3 treatments and 3 replications. The experiment was carried out semi-static. The solutions of 4-nonylphenol were renewed every 48 hours. Over the experiment, fish were maintained in 12:12 (Light:Dark) photoperiod and average temperature 13-15 °C. The physical and chemical water conditions properties of the test water are recorded in table 1. Before the experiment, a stock solution of 4-nonylphenol (Riedel, PESTANAL, formula:  $C_{15}H_{24}O$ , made in Germany), analytical standard, technical mixture and purity 98% was prepared by dissolving it in absolute ethanol. Along with the research done on the effects of 4-nonylphenol on the two species of the Acipenseridae by Dwyer et al. (2000) and the study related to evaluation of concentrations of 4-nonylphenol, octylphenol and bisphenol A in the southern coastal Caspian Sea (Anzali wetland) which was done by Mortazavi et al. (2012), in the present study, two sub-lethal concentrations for Iranian sturgeon (*Acipenser persicus*) was spotted: 40 and 70 µg/l and control in three replications. Therefore, 4-nonylphenol was added in appropriate volumes (40 and 70 µg/l) of 100%

ethanol (Merck, Germany) to reach nominal concentrations of 40 and 70 µg/l water and also, ethanol alone with concentration 70 µg/l was added in control to reach a concentration fixed (Dreze et al., 2000). For light microscopic studies, dead and deformities prelarvae in the three phases (10, 20 and 30 days post hatching) were fixed by immersion in Bouin fluid for 24 hour and then samples were transferred to ethanol 70%. These samples were dehydrated in ethanol series of ascending concentrations, embedded in paraffin and serially sectioned at 7 µm thickness on a Micro DS 4055 microtome. This research continued to the ten days post active feeding. The tissue sections were dried for 24 h and stained with haematoxylin - eosin (H&E), then different sections of each tissues were studied by light microscope (Pearse 1985). Histopathological changes of prelarvae from treatment groups were made by using the microscope connected with a computer image analysis system MultiScan. Then, they were photographed.

#### **Data analysis**

All data (mortality and abnormalities) in the three times periods (every period was ten days) were tested by SPSS (version 16). All data are expressed as the mean ± S.E.M. The data of mortality

were subjected to a two-way ANOVA ( $p < 0.05$ ). The abnormalities of the gill, liver and kidneys embryo were subjected to nonparametric analyses using a Kruskal-Wallis one way ANOVA on ranks followed by mann-whitny U test ( $p < 0.05$ ).

## RESULTS

The physical and chemical properties of the test water are presented in table 1 and the mortality of *Acipenser persicus* prelarvae of the three periods during this experiment are presented in table 2.

**Table 1: Physical and chemical properties of the test water**

Parameter	Volume
pH	8-8.5
salinity	0.5-0.9 mg/l
dissolved oxygen	$5.8 \pm 0.10$ mg/l
total hardness	$400 \pm 5.5$ mg/L

**Table 2 Percentage mortality of the *Acipenser persicus* prelarvae of the three time period development in the three concentration**

Period	Percentage mortality in three treatments		
	Control	40 $\mu$ g/l	70 $\mu$ g/l
1 <sup>st</sup> ten days	$0.6 \pm 0.3$	$1.3 \pm 0.3$	$2.3 \pm 0.3$
2 <sup>nd</sup> ten days	$2.3 \pm 0.3$	$3.3 \pm 0.3$	$7.0 \pm 0.5$
3 <sup>rd</sup> ten days	$8.3 \pm 0.8$	$18.3 \pm 0.8$	$21.6 \pm 1.7$

Values are given as mean  $\pm$  S.E.M.

The embryo of the *Acipenser persicus* exposed to sub-lethal concentrations of 4-nonylphenol during this period showed considerable degree of alteration in the tissues of the gill, kidney and liver. The histological alterations found in the gills of the prelarvae exposed to 4-nonylphenol included hyperplasia and severe lesions in the tissue of the gills, rupture of lamellar epithelial (fig. 2c and fig. 3c). The main alterations found in the liver tissue were vacuolated hepatocytes, central necrosis and cell degeneration (fig. 5c and fig. 6c). The most alterations found in the kidney were hypertrophied nucleus cell of tubules (fig. 8b) and degeneration of the bowman's capsule (fig. 8c and fig. 9c). According to table 2 in ten days of the

tertiary, the maximum mortality of the prelarvae's *Acipenser persicus* was  $21.6 \pm 1.7$  in the 70  $\mu$ g/l. Therefore, the maximum of deformities cited above occurred at this period.

## DISCUSSION

The study of the relation to alkyphenols concentrations and its effects of the environment and living things is negligible in the southern part of the Caspian Sea. Perhaps, the latest research was done by Mortazavi and his colleagues in the 2012. In the aforesaid research, concentrations of the alkyphenol compounds such as 4-nonylphenol in Anzali wetlands in Iran was estimated. The concentrations of 4-nonylphenol was  $29\mu$ g/g dry weight in that region. On the

other hand , the existence of endangered species in the southern part of the Caspian Sea like *Acipenser persicus* , the necessity of paying attention to any kind of factors which cause to negative effects on this species was showed. Therefore , in the present study and the semi-static experimental condition two sub-lethal concentrations ( 40 and 70  $\mu\text{g/l}$ ) was pointed. According to table 2 , increase of percentage of the mortality along with increase of the age of embryo happened during 30 days . So, the percent of the mortality mean was raised to  $21.6\pm 1.7$  in the 70  $\mu\text{g/l}$  ; because the sensitivity of prelarvae of the sturgeon fish was raised to the mount of the age prelarvae in the phenolic compounds (Dettlaff et al. , 1993) . In the environment with the kinds of the pollutions , one of the best ways to check the health of the fish is the survey of the histopathological changes of the organs that can show the negative effects of excessive accumulation of the pollutants in the environment (Van der Ost et al. , 2003). In the present study , gill , liver and kidney's embryo of the *Acipenser persicus* are biomarkers . Because studying the specific tissue is one of the advantages of the biomarkers in the animals , survey histological alteration are very easy in fish (Fanta et al. , 2003). In the histopathological study

on the gill , liver and kidney in the embryo of the *Acipenser persicus* no changes were observed on the control , 40 and 70  $\mu\text{g/l}$  during the first ten days but continuity of the development of the embryo in the second ten days , hyperplasia on the lamella of the gills was observed in the 70  $\mu\text{g/l}$  but no changes on the control and 40  $\mu\text{g/l}$  (fig. 1,2,3 ) . In the hyperconcentration of the pollutions , hyperplasia is a defense mechanism ; this position causes the increase in distance external environment and the circulatory gills ; Therefore , a kind of dam is created against the penetration of the pollutions (Carmono et al. , 2004) . So , during the tertiary of ten days this position was continued for gills . The liver had not any histological alteration in the first ten days but according to figure 5 and 6 , vacuolated hepatocytes and central necrosis was observed in the second and tertiary ten days , especially on the 70  $\mu\text{g/l}$  . The alterations of the liver tissue usually happen in the following penetration of the phenol compounds (Abdel-Hameid , 2007). Also , Carbohydrates are usually the first energy source particularly in case of stress . Therefore , exposure to the kind of pollutants causes reductions in muscles and liver glycogen content (Camargo et al. , 2007) . The teleostean

kidney is one of the first organs to be affected by contaminant in the water (Thopon et al. ,2003) . The most common alterations found in the kidney of fishes exposed to water contamination are tubule degeneration and changes in the corpuscle such as dilation of capillaries and reduction of Bowman's space (Takashima & Hibiya , 1995) . In the present study , the alterations in kidney were hypertrophied nucleus cell of tubules and degeneration of the bowman's capsule , especially on the 70 µg/l and the second and tertiary ten days , too ( fig. 8b and fig. 9c ) . In conclusion the present study indicate that alkyl phenols such as nonylphenol as the aquatic pollution , produce many adverse effects on fish and aquatic ecosystems when being chronically exposed , even at a small concentration . Also, it showed that histopathological alterations are useful biomarkers for environmental contaminations . The histopathological impacts observed in gill , kidney and liver of the fish embryo in the present study indicate that the fish were responding to the direct effects of the contaminants as much as to the secondary effects caused by stress. Of course , it is possible that the other factors influence the deformities of the fish embryo sturgeon . Because the

long period of the puberty and reproduction ( 8 to 18 years ) in the *Acipenser persicus* (Chebonov et al. , 2011) , survey effects of the pollution on the *Acipenser persicus* will be a costly and difficulty work in the natural environment but may to point that a research has been done by jamshidi and his colleagues (2012) which was related to the effects of nonylphenol on production and changes of plasma vitellogenin protein in the Persian sturgeon ; according to that research adverse effects of nonylphenol on the gonadal development is exhibited. Unfortunately , there isn't any accurate information related to concentration of the 4-nonylphenol and negative effects on the environment and living things in the southern part of the Caspian Sea till now ; therefore guarding researches are necessary in this region .

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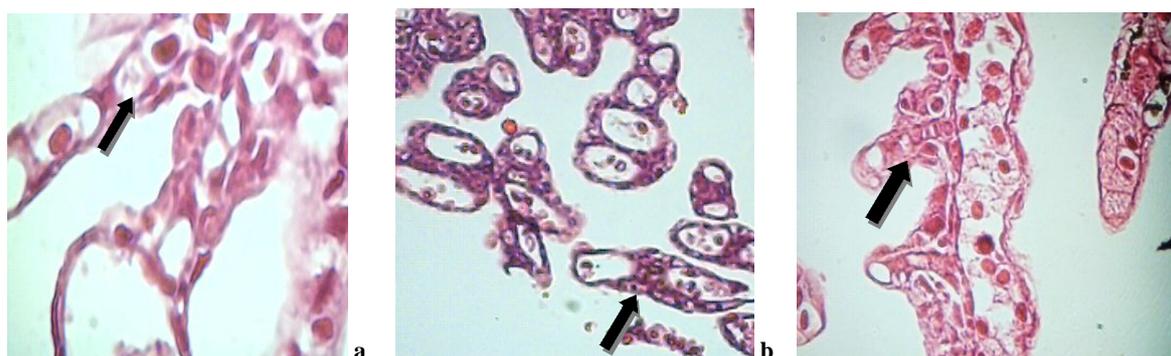


Figure 1: photomicrographs of the gill of the prelarvae's *Acipenser persicus* in the first ten days . (a) control ; ( b) 40 µg/l and (c) 70 µg/l of 4-nonylphenol . pillar cells (black arrow). H&E stained.

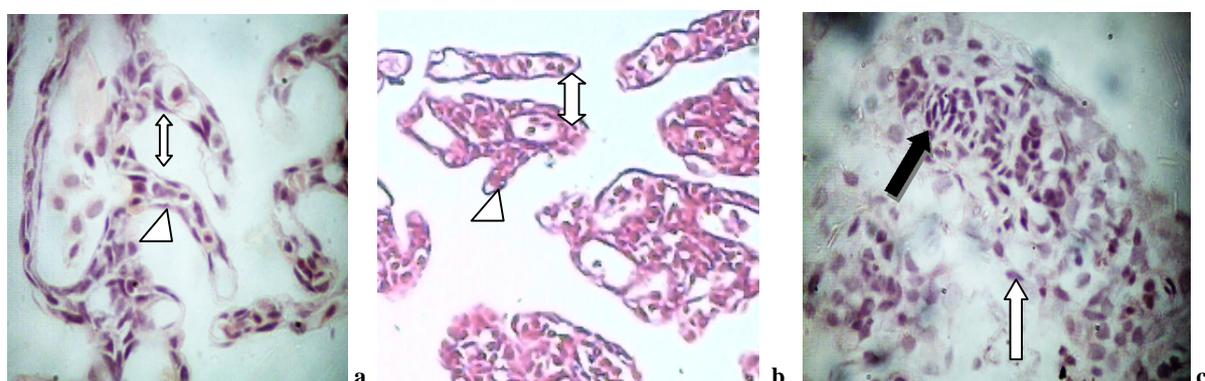


Figure 2: photomicrographs of the gill of the prelarvae's *Acipenser persicus* in the second ten days . (a) control ; ( b) 40 µg/l and (c) 70 µg/l of 4-nonylphenol . the water channel between gill filament ( bilateral arrow ), a pillar cell (arrowhead) , hyperplasia of the epithelial cells ( black arrow ) and epithelial rupture ( white arrow ). H&E stained

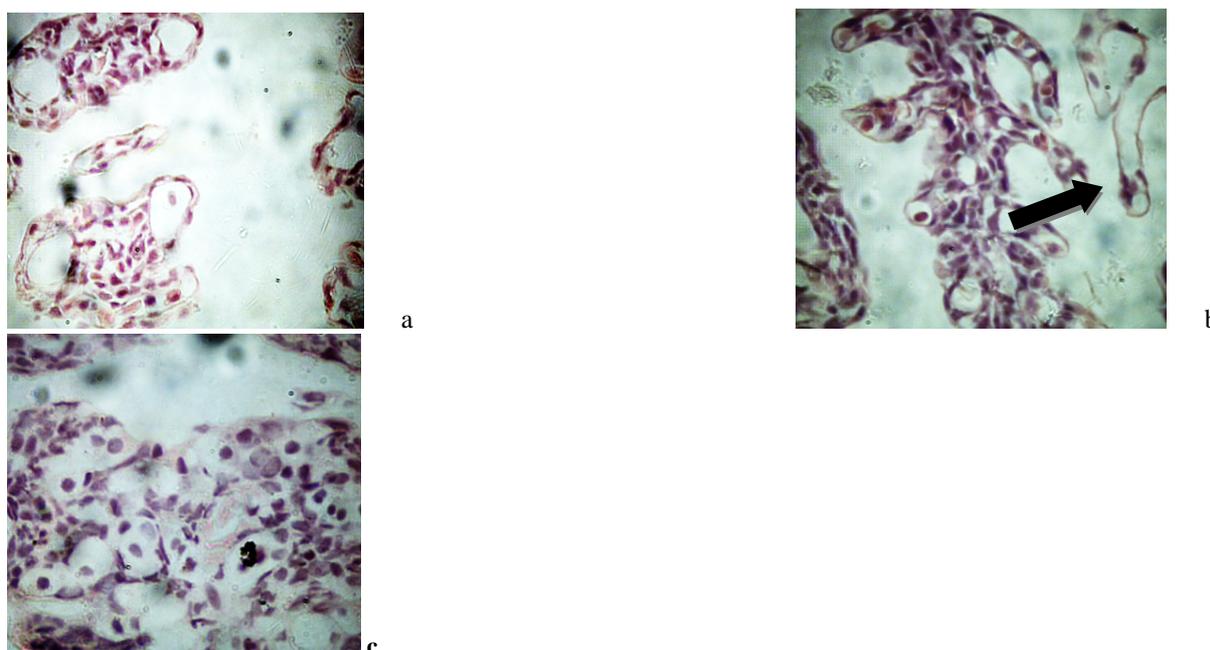


Figure 3. photomicrographs of the gill of the prelarvae's *Acipenser persicus* in the tertiary ten days . (a) control ; ( b) 40 µg/l and (c) 70 µg/l of 4-nonylphenol . hyperplasia of the epithelial cells ( black arrow ). H&E stained .

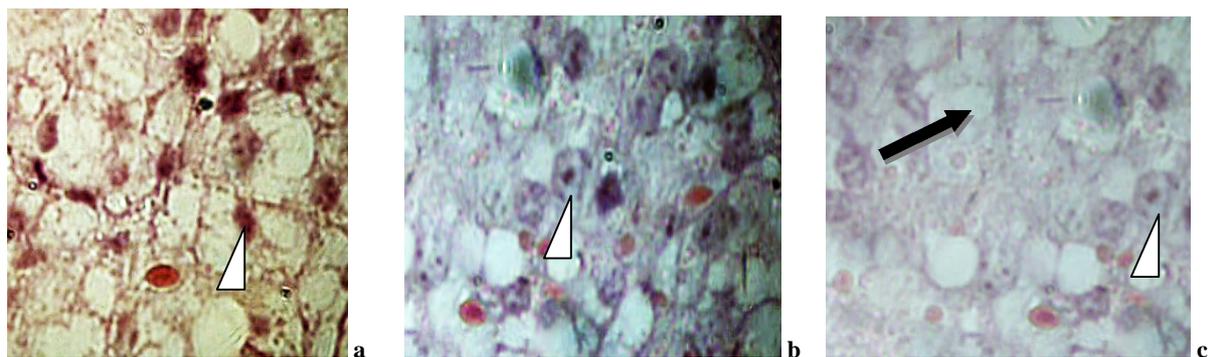


Figure 4. photomicrographs of the liver of the prelarvae's *Acipenser persicus* in the first ten days . (a) control ; ( b) 40 µg/l and (c) 70 µg/l of 4-nonylphenol . hepatocyte (arrowhead), vacuolated tissue ( black arrow ) . H&E stained .

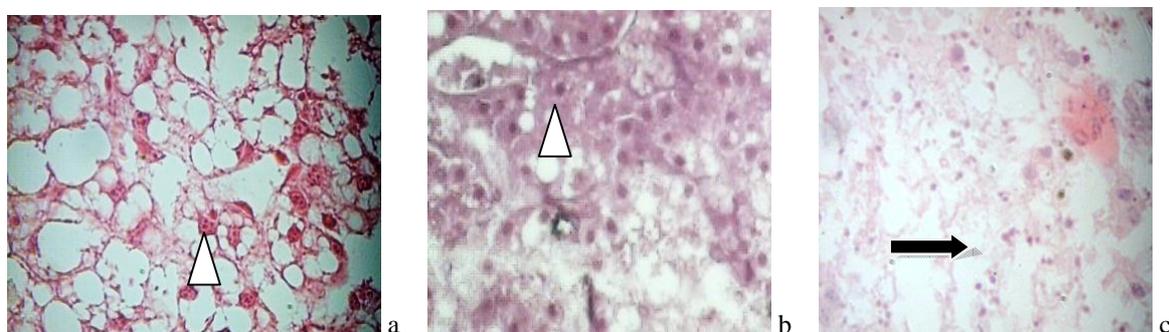


Figure 5. photomicrographs of the liver of the prelarvae's *Acipenser persicus* in the second ten days . (a) control ; ( b) 40 µg/l and (c) 70 µg/l of 4-nonylphenol . hepatocyte (arrowheads) , vacuolated and tissue necrosis ( black arrow ) . H&E stained

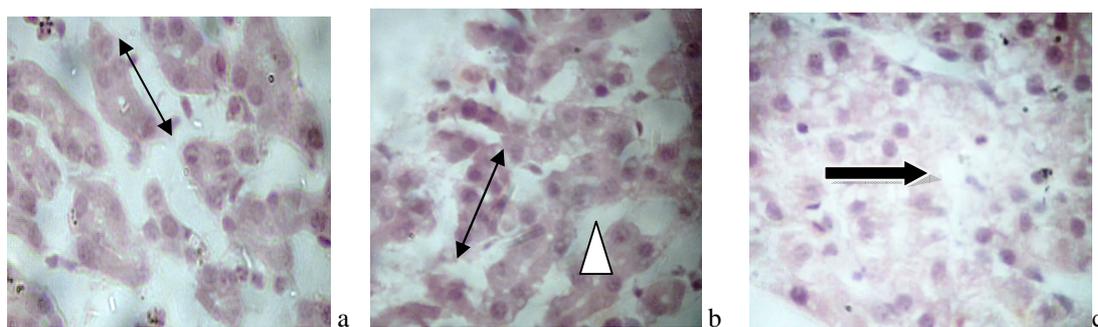


Figure 6. photomicrographs of the liver of the prelarvae's *Acipenser persicus* in the tertiary ten days . (a) control ; ( b) 40 µg/l and (c) 70 µg/l of 4-nonylphenol . hepatocyte discs ( bilateral arrows) , vacuolated tissue (arrow head) , tissue necrosis ( black arrow ) . H&E stained .

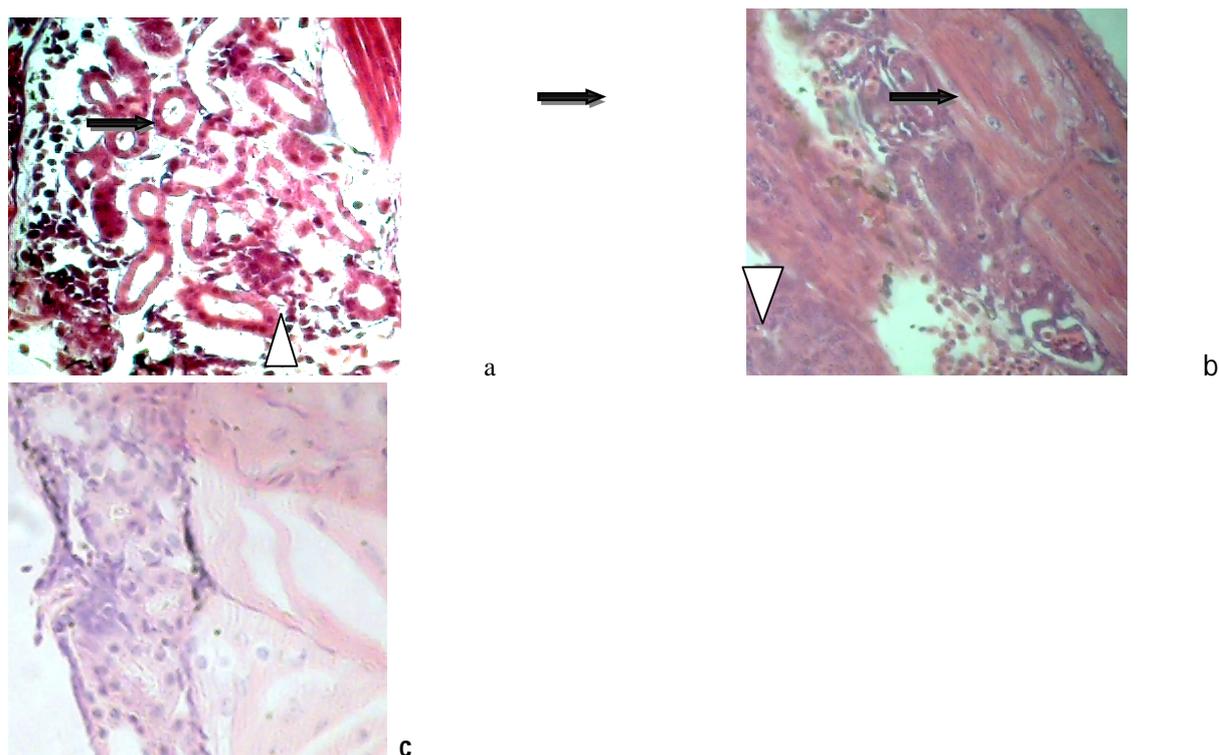


Figure 7. photomicrographs of the kidney of the prelarvae's *Acipenser persicus* in the first ten days . (a) control ; (b) 40 µg/l and (c) 70 µg/l of 4-nonylphenol . Glomerulus and the bowman's space (arrowhead) , tubule (black arrow) . H&E stained .

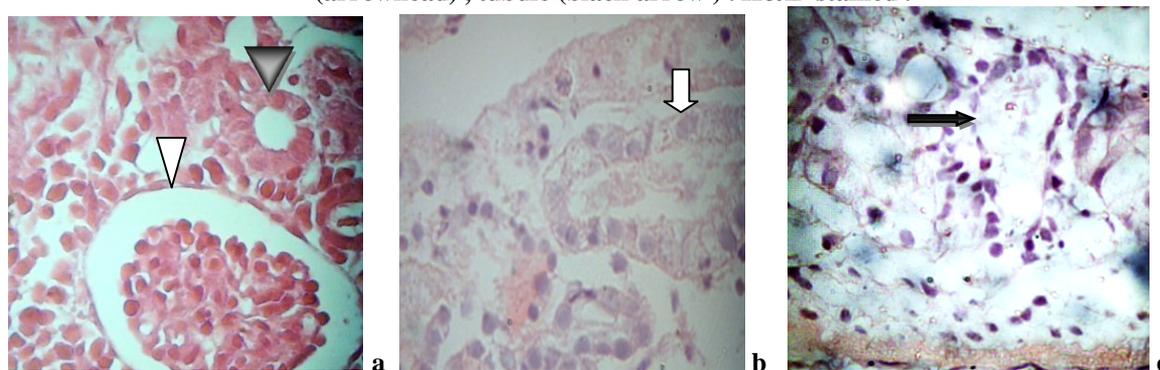


Figure 8: photomicrographs of the kidney of the prelarvae's *Acipenser persicus* in the second ten days . (a) control ; (b) 40 µg/l and (c) 70 µg/l of 4-nonylphenol . Glomerulus and the bowman's space (arrowhead) , tubule (black arrowhead) , tubule cells with hypertrophied nucleus (white arrow) , degeneration of the bowman's capsule and glomerulus (black arrow) . H&E stained

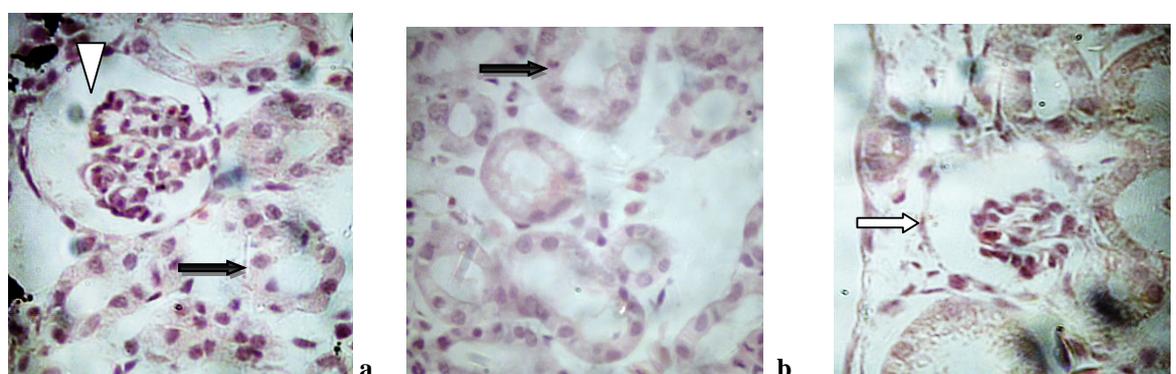


Figure 9: photomicrographs of the kidney of the prelarvae's *Acipenser persicus* in the tertiary ten days . (a) control ; (b) 40 µg/l and (c) 70 µg/l of 4-nonylphenol . Glomerulus and the bowman's space (arrowhead) , tubule (black arrow) , degeneration of the bowman's capsule (white arrow) . H&E stained .

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