Effect of Aqueous Solutions of Pomegranate Peel on Some Heavy Metals in Grey Mullet (Mugilcephalus Linnaeus, 1758)
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Abstract:
Water and grey mullet (Mugilcephalus) samples were collected from Mediterranean Sea, Manzalh Lake, and a private fish farm was contaminated with Cd, Hg and/or Pb. The Pb concentration was at highest in the Lake water. Whereas, Cd and Hg levels were the highest in the fish farmwater. Generally, the level of water Pb was higher than water Cd than water Hg, respectively. There were significant differences among tested locations, as well as the treatment period in concentration of the three heavy metals tested in the fish samples. Lake's fish samples were the highest in Pb concentration, but fishfarm samples were the highest in Cd levels. However, Hg level in the sea fish samples was the highest.

Pomegranate significantly reduced the level of the three heavy metals in the fish flesh samples. Moreover, prolonged treatment period (20 and 30 min.) reduced the heavy metals level, particularly Pb and Cd. Thus, it is possible to recommend the importance of soaking fish before cooking in the water extract of pomegranate peel (1 g pomegranate powder / 100 g fish) for 20 or 30 minutes to reduce the concentration of these dangerous pollutants in fish flesh and make them safe for human consumption.

Keywords: Mullet, Bury, sampling locations, pomegranate peel, Heavy metals.

Introduction:
Egyptians prefer to consume fish than of white meat (poultry, ducks, geese, pigeons and chickens) and red meat (from farm animals, such as beef, buffafo, camels, goats and sheep). The Egyptians consumed about 1.8 million tons annually, although the fish produced is 1.4 million tons, of which about 70% (705 thousand tons of farmed fish). For that reason, Egypt imports fish to overcome supply shortages (Abdelhamid et al., 2014b). The annual production of Egyptian fish in 2017 amounted to about 1.7 million tons, of which 210 thousand tons of cultured mullet (while the production of both the Red Sea and the Mediterranean does not exceed in the production of 7.3%). However, Egypt ranks ninth in the global aquaculture ranking (FAO, 2017).
Damietta governorate is characterized by its diverse fisheries; it has freshwater fisheries (Nile River), brackish (Manzalh Lake), and maritime (Mediterranean Sea). Therefore, the population of Damietta often consumes fish more than other governorates; however, increasing environmental pollution, whether locally or globally, leads to increase pollution of fish and thus is transmitted to the consumer, which harms public health and thus affects the economic activity in Egypt (Alsaiedy, 2002).

The most hazardous pollutants that move from water to fish and thus to the consumer are the heavy elements, this has led to an increase in the number of people with liver cancer in Egypt, where the rate of patients with the disease rose from 4% in 1993 to 11% in 2009. Moreover, the mortality rate among Egyptians also increased from 2.8 / 100,000 in 1990 to 10 / 100,000 in 2015. In addition to the high incidence of other diseases related to pollution of fish through water pollution, and thus increased the interest of researchers in the field of nutrition and food science to find the link between diseases and food contamination, especially fish (Samirabdelghaffar.com/2016).

Consuming of fish products may ameliorate disorders involving thrombosis, blood pressure, arthritis, asthma, arthrosclerosis, tumor growth, etc. However, uncontrolled rearing, handling and processing may result in negative impacts to health. Pollution of fish rearing water may be harmful for fish and human health (Abdelhamid, 2009a, b, & c, and Abdelhamid et al., 2014a & b). Grey mullet (Mugilcephalus) is one of the most popular fish species in most of the Mediterranean basin countries including, Egypt. M. cephalus is a saltwater fish, as well as it can be cultured in freshwater with heavier yield than from marine water, with a greater return than marine water (Abdelhamid, 2003). The per person consumption of farmed fish will be more than marine water fish consumption (Koeleman, 2014).

Fish ponds receiving nutrients derived from treated night soil were less contaminated than ones to which untreated night soil was applied, and the fish reared in them were of superior quality (Ling et al., 1993). Therefore, the present research aimed to study the possibility of reducing or overcome the heavy metal contamination of grey mullet (M. cephalus) by using pomegranate peel before its cooking, mainly soaking solutions of pomegranate peel (parsley) seed powders on some heavy metals' (Pb, Cd, and Hg) concentrations before and after the treatments, by soaking fish samples for three periods (10, 20, and 30 min.).
Materials and Methods:

This study was carried out under the cooperation between Department of Home Economics, Zagazig Faculty of Specific Education, Zagazig University and Department of Animal Production, Mansoura Faculty of Agriculture, Mansoura University during 2017 – 2018. The study aimed to evaluate the effect of preparing mugil fish before cooking using natural agent, i.e. pomegranate peel (parsley) seed powders on some heavy metals' (i.e. Pb, Cd, and Hg) concentrations before and after the treatments, by soaking fish samples for three periods (10, 20, and 30 min.).

Water samples:

Three water samples from each location [ the Mediterranean Sea (from Gamsa-elbalad about 500 meters from the land), Manzalh Lake (pond of gait el nasara) and fish farm (Hassan Ghanem farm-El Deba) ] from Damietta governorate were collected during July 2017. The water samples were frozen, and then lead, cadmium and mercury were measured in these samples. The average for each sampling location was then calculated.

Fish samples:

Three fish samples of grey mullet (M.cephalus) caught from each location (as in water sampling) were bought from the local market, i.e. Mediterranean Sea (average weight 200g), Manzalh Lake (average weight 200g), and a private fish farm (average weight 250 g) in Damietta governorate during July 2017.

Preparation of fish samples:

Fish samples were cleaned by removing the scales, gills and eviscerated by removing the complete gut. The fish samples were divided into three groups (according to its sampling location) to be treated before cooking with commercial powder of pomegranate (Punicagranatum) peel (external and internal skins). This natural agent was purchased from the local market for spices and medical herbs in powder form.

Fish from each sampling location were rather divided into four parts, the first part was not soaked in the preparing solutions, Whereas the second, the third and the fourth parts were soaked in the preparing solutions with concentration of 1g powder / 100 g fish for 10, 20 and 30 minutes, respectively as the following:

1- Mugil fish from marine without soaking.
2- Mugil fish from marine have been soaked for 10 minutes.
3- Mugil fish from marine have been soaked for 20 minutes.
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4- Mugil fish from marine have been soaked for 30 minutes.
5- Mugil fish from Manzalh Lake without soaking.
6- Mugil fish from Manzalah Lake have been soaked for 10 minutes.
7- Mugil fish from Manzalah Lake have been soaked for 20 minutes.
8- Mugil fish from Manzalah Lake have been soaked for 30 minutes.
9- Mugil fish from a farm without soaking.
10- Mugil fish from a farm have been soaked for 10 minutes.
11- Mugil fish from a farm have been soaked for 20 minutes.
12- Mugil fish from a farm have been soaked for 30 minutes.

After the treatment, fish were frozen to be easier to cut the tissues for heavy metals analysis.

Fish and water analyses:

Fish samples were transported to the laboratory in an ice-box. The experimental fish specimens were cooled for as hot time then kept frozen (till analysis) to avoid any loss of compounds and to be easy to cut the frozen tissues. The fish were taken from the freezer and kept at room temperature until they were partly thawed. The fish were put on a clean plastic plate, the skin was removed using a knife, and then a flesh sample was tweezed from the lower part of the dorsa–lateral tissue (Dybern, 1983).

The analyses of heavy metals in both water and fish samples were done in the Zagazig University Agricultural Research and Experimental Center, Faculty of Agriculture Central Laboratory using Atomic Absorption Spectrophotometer (AAS, model 2380, from Perkin Elmer Company, USA).

Statistical analysis:

The obtained numerical data were statistically analyzed using SAS (2006) for analysis of variance. Differences between comparisons among treatment mean were made by using Duncan multiple ranges test (Duncan, 1955).

Results and Discussion:

Heavy metals of fish rearing water:

Responsible authorities have set some permissible limits for most heavy metals, especially that which classified as very toxic, Pb, Cd and Hg in the fish rearing water as 0.10 mg / L of Pb and 0.01 mg / L of Cd. Moreover,
the Sea water did not contain Cd, but contains 0.0003 mg/L Hg and 0.004 – 0.005 mg/L Pb (Abdelhakeem et al., 2002).

Abdelhamid and El-Ayouty (1991) proved that the presence of non-organic lead in fish breeding water makes it poisonous and may lead to fish mortality or the emergence of certain diseases such as, lack of fish protein, increased body fat, kidney and gastrointestinal congestion and bleeding, the risk increases by increasing pollution.

Data of the heavy metals' analysis (mg/L) in the fish rearing water from the sampling locations (sea, lake, and farm) for lead (Pb) were 0.376, 0.547, and 0.132; for cadmium (Cd) 0.025, 0.0000, and 0.049; and for mercury (Hg) 0.380, 0.001, and 0.009 mg/L, respectively as shown in Table (1). These results mean that Pb concentration was at highest in the lake water followed by the sea water then the farm water. Whereas, Cd was absent from the lake water, but highest level was found in the farm water followed by the sea water. However, Hg level was at highest in the sea water, followed by the farm water and at least in the lake water.

On other hand, the farm water was polluted with the highest level (mg/L) of Cd (0.049), the sea water was the highest concerning of Hg (0.380), whereas the lake water was the highest concerning of Pb (0.547 mg/L). Generally, the level of water Pb was higher than water Hg than water Cd, respectively.

According to World Health Organization (WHO, 1984), the limits on lead, cadmium and mercury in fish rearing waters were 0.05, 0.005 and 0.001 mg/L. The standard specifications for water suitable for fish farming are described as heavy metals as less than 0.02 mg/L Pb, 0.0005 mg/L Cd, less than 0.002 mg/L Hg (U.S. EPA, 1974). Consequently, the water from the tested locations contained more Pb than the acceptable level of Pb. Also, farm and Sea waters were not save concerning their content of Cd. El-Safy and Al-Ghannam (1996) registered higher concentrations for the three heavy metals in Lake Manzalh water than our results, with higher concentrations in 83% of the water samples than the permissible limits.

Manzalh Lake is exposed to various forms of pollution that adversely affect its water environment. Whether from sewage or agricultural or industrial pollution, the sampling capacity of Manzalh Lake greatly affects the level of heavy metals in its waters. Moreover, heavy metals contaminated negatively affect the amount of fish production and health of fish (Magouz et al., 1996).

Abdelhamid and Gawish (1998) and Abdelhamid et al. (2013a &b) reported that there are significant differences in levels of heavy metals in
water due to sampling sites. Salem (2003) found that the heavy metals pollution reduces the area of fish tissues. Abdelhamid et al. (2013a) gave the following order of heavy metals concentration in water Cd>Pb. Abdelhamid et al. (2013b) calculated significant correlations among heavy metals' level in fish.

Table 1: Mean* values of heavy metals Pb, Cd, and Hg (mg / L) in the water of Mediterranean Sea, Manzalh Lake, and water of the fish farm at zero time

<table>
<thead>
<tr>
<th>Source of water</th>
<th>Pb</th>
<th>Cd</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mediterranean Sea</td>
<td>0.376 ± 0.008^b</td>
<td>0.025 ± 0.001^b</td>
<td>0.380 ± 0.012^a</td>
</tr>
<tr>
<td>Manzalh Lake</td>
<td>0.547 ± 0.015^a</td>
<td>0.000 ± 0.000^c</td>
<td>0.001 ± 0.000^b</td>
</tr>
<tr>
<td>Fish farm</td>
<td>0.132 ± 0.006^c</td>
<td>0.049 ± 0.004^a</td>
<td>0.009 ± 0.000^b</td>
</tr>
</tbody>
</table>

*Mean values of three replicate samples ± Standard Deviation

a, b and c: means in the same column with different small letters are significantly differ (P ≤ 0.05)

Heavy metals of Mugilcephalus' smuscles:

Pollution is one of the most serious problems faced by living organisms and the environment as well, its forms and sources varied, the impact of pollution extends to the lives of the living organisms and their related health, food, economy and production (Abdelhamid, 1999, 2000, 2001 and 2009c). Therefore, in order to protect the environment and living organisms, the responsible authorities should take legal measures to treat all wastewater prior to entering the water bodies. (Abdelhamid et al., 2007).

Heavy metals analysis of M. cephalus revealed presence of significant differences among sampling locations, as well as the treatment period in concentration of the tested three heavy metals. Samples of M. cephalus collected from Sea, Lake, and Farm were analyzed to determine the Pb, Cd, and Hg in their tissues. Data presented in Table (2) revealed presence of significant differences (P ≤ 0.05) among sampling locations, as well as at zero time in concentration of the three heavy metals in the fish tissues.
Lake fish samples were contaminated with higher levels of Pb > Sea > farm samples.

Whereas, the farm fish samples were more contaminated with Cd > Sea > Lake samples. Yet, Sea fish samples reflected the highest Hg concentrations > farm > Lake samples. Regardless to sampling locations, the levels of Pb > Hg > Cd (Table 2). Lake samples contamination levels took the descending order Pb, Hg, and then Cd, being 1.304, 0.002, and 0.000 mg / L, respectively. Sea fish flesh samples reflected the contamination levels of heavy metal as Pb, Hg, and Cd being 0.547, 0.215 and 0.036 mg / L, respectively. Finally, farm fish flesh samples reflected the descending order of heavy metal contamination levels as Pb, Cd, and Hg, being 0.287, 0.196 and 0.019 mg / L, respectively. In this respect, El-Safy and Al-Ghannam (1996) registered higher concentrations of the three heavy metals in Manzalh Lake fish than our results, with higher concentrations in 6.94, 28.7, and 100% of the fish samples than the permissible limits of Pb (1.00 mg / L), Hg (0.50 mg / L), and Cd (0.10 mg / L), respectively according to EGASQC (1991).

Table 2: *Mean values of heavy metals Pb, Cd, and Hg (mg / L) in the fish tissues from Mediterranean Sea, Manzalh Lake, and the fish farm at zero time

<table>
<thead>
<tr>
<th>Heavy metal (mg / L)</th>
<th>Pb (-)</th>
<th>Cd (-)</th>
<th>Hg (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of fish</td>
<td>Zero time</td>
<td>Zero time</td>
<td>Zero time</td>
</tr>
<tr>
<td>Mediterranean Sea</td>
<td>0.547±0.074 b</td>
<td>0.036±0.009 b</td>
<td>0.215±0.043 a</td>
</tr>
<tr>
<td>Manzalh Lake</td>
<td>1.304±0.112 a</td>
<td>0.000±0.000 c</td>
<td>0.002±0.001 c</td>
</tr>
<tr>
<td>Fish farm</td>
<td>0.278±0.098 c</td>
<td>0.196±0.071 a</td>
<td>0.019±0.007 b</td>
</tr>
</tbody>
</table>

* Mean value of three replicate samples ± Standard Deviation  
  a, b and c: Mean with different letters in the same column are significantly differ (P≤0.05).

Similarly, with the current obtained findings Abdelhakeem et al. (2002) found the tolerance levels of Pb and Cd 2.00 and 0.50 mg / L in fresh fish body. Abdelhamid et al. (2000b) reported that the lowest accumulation of heavy metals in fish is found in the edible portions. Abdelhamid et al. (1997) recorded that, there are significant differences in the levels of heavy metals.
metals in the fish tissues according to the sampling sites. In addition, the levels of heavy metals in fish tissues were higher than those found in fish farming water.

The levels of Cd and Pb in the muscular system of fish were above the permissible levels. In addition, *Lisa ramada* and *Sparus aurata* were less contaminated than *M. cephalus*.

**Abdelhamid and Gawish (1998)** showed that the levels of heavy metals in crab and shrimp are in ascending order Pb ≥ Hg ≥ Cd. They found that 100% of the samples were rejected for the higher contents of cadmium than the level allowed, 47.6% and 70% are not acceptable for high mercury content, in addition to 35.7% and 42% due to high lead concentrations. Therefore, they recommend that sources of pollution should be treated before they reach the water bodies. Until this is done, the consumption of this polluted seafood should be regulated to reduce public health risk exposure. However, mineral levels are associated with salinity changes due to drainage (Radwan, 2000). The latest author also reported an effect of sampling locations on heavy metals' levels in fish. Moreover, **Abdelhamid et al. (2013a)** gave the following order of heavy metals in fish Pb > Cd. They calculated significant correlations among heavy metals' concentrations in water and fish from one side and chemical composition of the fish. **Gawish and Hosni (2017)** found that Pb level in fish tissues was higher than Cd. They found also correlations between heavy metals' level and chemical composition of the fish tissues. Their findings of heavy metals' concentrations were higher than those found at our study. Generally, the treatment reduced the heavy metals concentration comparing with zero time before treatment. Moreover, prolonged treatment period (20 and 30 min.) generally reduced (P ≤ 0.05) the heavy metals level, particularly in Pb and Cd (Table 3).

**Table 3: Mean* values of heavy metals Pb, Cd, and Hg (mg / L) in the fish tissues from Mediterranean Sea after effect of soaking in natural agents of Pomegranate at zero, 10, 20 and 30 min**

<table>
<thead>
<tr>
<th>Location</th>
<th>Treatment</th>
<th>Period min.</th>
<th>Pb mg / L</th>
<th>Cd mg / L</th>
<th>Hg mg / L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mediterranean Sea</td>
<td>Pomegranate</td>
<td>0</td>
<td>0.547±0.148a</td>
<td>0.036±0.017a</td>
<td>0.215±0.086a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.142±0.056b</td>
<td>0.021±0.006b</td>
<td>0.189±0.008b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>0.008±0.132c</td>
<td>0.013±0.013c</td>
<td>0.101±0.039c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>0.006±0.126c</td>
<td>0.003±0.015d</td>
<td>0.090±0.088d</td>
</tr>
</tbody>
</table>

*Mean value of three replicate samples ± Standard Deviation
a, b, c and d: Mean with different letters in the same column are significantly differ (P≤0.05).
Lake fish samples levels took the descending order Pb, Hg, and then Cd, being 1.304, 0.002, and 0.000 mg / L, respectively. It was clear that increasing the treatment period had decreased the residues of Pb in the fish flesh from Lake. The effect of the treatment agent (Pomegranate) was equal in removing Hg at all periods of soaking that pomegranate removed Hg completely in the three periods; whereas, Cd was absent as shown in Table 4.

Table 4: Mean* values of heavy metals Pb, Cd and Hg (mg / L) in the fish tissues from Manzalh Lake after of soaking in natural agents of Pomegranate at zero, 10, 20 and 30 min

<table>
<thead>
<tr>
<th>Location</th>
<th>Treatment</th>
<th>Period min.</th>
<th>Pb mg / L</th>
<th>Cd mg / L</th>
<th>Hg mg / L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manzalh Lake</td>
<td>Pomegranate</td>
<td>0</td>
<td>1.304±0.224ª</td>
<td>0.000±0.000</td>
<td>0.002±0.001ª</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.100±0.100b</td>
<td>0.000±0.000</td>
<td>0.000±0.000ª</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>0.064±0.093c</td>
<td>0.000±0.000</td>
<td>0.000±0.000ª</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>0.059±0.159c</td>
<td>0.000±0.000</td>
<td>0.000±0.000ª</td>
</tr>
</tbody>
</table>

* Mean values of three replicates samples ± Standard Deviation
a, b and c: Mean with different letters in the same column significant differ (P≤0.05).

Farm fish flesh samples reflected the descending order of heavy metal contamination levels as Pb, Cd, and Hg, being 0.278, 0.196, and 0.019mg / L, respectively as shown in Table (5). It was clear from Table (5) that increasing the treatment period had decreased the residues of all tested heavy metals in the fish flesh from farm. Pomegranate removed the Pb completely from the fish flesh from the farm. While pomegranate removed Cd with increased period of soaking to 20 minutes, whereas the increase in the period of soaking in pomegranate led to the removal of Hg. Generally, all treatment periods reduced the heavy metals' concentrations than before treatment.

Use dried sewage sludge (DSS) containing Cu, Cd, Pb and P is higher than in the commercial diet, in feeding farmed fish (such as Nile tilapia, African catfish, silver carp, and common carp) resulted in fish containing levels of Pb and Cd more than control fish. Thus, it is recommended not to use DSS, which is widely used in Kafr El-Sheikh governorate. In addition, it is recommended to give more attention to the quality of water and food used in fish farming to provide safe products for human consumption Abdelhamid et al. (2014 a). Moreover, Abdelhamid et al. (2013 a)
reported that at Ashtom El-Gamil (Manzalh) lakes and gardens (protected areas) are contaminated with heavy metals. These authors revealed significant differences (P ≤ 0001) with respect to the levels of heavy metals tested in water, sediment or fish, the sampling locations and seasons, as well as their reactions. The level of elements took the descending order Cd ≤ Zn ≤ Cu ≤ Pb ≤ Fe in the sediment, Cu ≤ Fe ≤ Pb ≤ Cd ≤ Zn in the water, and Cd ≤ Cu ≤ Zn ≤ Pb ≤ Fe in the fish tissues. Some important correlations have been calculated between heavy metals (in sediment, water, and fish) and chemical composition of fish.

Manzalh Lake suffers from industrial and agricultural pollution and continuous sanitation, which negatively affects its water environment, where liquid waste discharges from industrial settlements with high population density. Heavy metals accumulate in the water and move into the fish accumulating in it, so studies should be done to follow the level of heavy elements in water and fish, and finding safer economic ways to remove heavy metals from polluted water.

Heavy metals (Cd, Pb, Cu, Zn, and Fe) were identified in gill and muscle of Tilapia nilotica in samples taken from the southern part of Lake Manzalh to assess the extent of contamination of lake water and fish by those toxic metals. Lead levels were higher than the maximum allowed for human consumption. That is, the levels of heavy metals observed in water and fish samples can be considered to represent a risk to the consumer. Therefore, many measures must be taken and some laws should be enacted to protect the lake and its aquatic life as well as to protect the consumer (EL-Shafei, 2015).

Table 5: Mean* values of heavy metals Pb, Cd, and Hg (mg / L) in the fish tissues from the farm fish after soaking in natural agents of Pomegranate at zero time, 10, 20 and 30 min

<table>
<thead>
<tr>
<th>Location</th>
<th>Treatment</th>
<th>Period min.</th>
<th>Pb mg / L</th>
<th>Cd mg / L</th>
<th>Hg mg / L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish farm</td>
<td>Pomegranate</td>
<td>0</td>
<td>0.278±0.197ª</td>
<td>0.196±0.141ª</td>
<td>0.019±0.013ª</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.005±0.005ª</td>
<td>0.100±0.000ª</td>
<td>0.005±0.004ª</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>0.000±0.000ª</td>
<td>0.000±0.000ª</td>
<td>0.003±0.001ª</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>0.000±0.000ª</td>
<td>0.000±0.000ª</td>
<td>0.000±0.000ª</td>
</tr>
</tbody>
</table>

* Mean values of three replicates samples ± Standard deviation

a, b and c: Mean with different letters in the same column are significantly differ (P ≤ 0.05).
Results in Table (6) shows in general that the overall mean value of Pb was > Hg > Cd, lake fish samples were more contaminated with Pb than sea and farm samples, being 1.304, 0.547, and 0.278 mg / L, respectively. However, farm fish samples were more contaminated with Cd than Sea and Lake ones, being 0.196, 0.036, and 0.000 mg / L, respectively. Yet, samples of Sea fish flesh were more contaminated with Hg than farm and lake samples, being 0.215, 0.019, and 0.002 mg / L, respectively. However, the overall means of heavy metals' concentrations in the sea fish samples were 0.547, 0.215, and 0.036 mg / L of Pb, Hg and Cd respectively, Lake samples contamination levels took the descending order Pb, Hg, and then Cd, being 1.304, 0.002, and 0.000 mg / L, respectively.

Finally, farm fish flesh samples reflected the descending order of heavy metal contamination levels as Pb, Cd, and Hg, being 0.278, 0.196, and 0.019 mg / L, respectively. On the other hand, the overall means of the contamination levels of Pb were significantly higher in lakes fish flesh samples than in sea and farm samples. Cadmium level was higher in farm samples than sea and lake samples. Yet, the Hg concentration in fish flesh sample from the sea was higher than those in fish samples belonging to farm and lake. It is known that heavy mineral content in the soil is affected by the sampling location (El-Ayoty et al., 1987).

Heavy metals are often found in naturally occurring foodstuffs (Abdelhamid et al., 1992) and also in soil, water and fish (Abdelhamid et al., 1997). Therefore, this pollution affects not only the survival and health of fish (Abdelhamid et al., 2000 a & b, 2006a; 2014 a, b, &c; and Abdelhamid and Ibrahim, 2003), but also affects human health (Shata, 1996). Where water pollution with heavy metals leads to about thirty-two serious diseases such as weight loss, alkalinity, parkinsonia, epilepsy, high toxic subs., fatigue, fetal damage, carcinoma abdominal pain, abdominal pain, myopathy, bronzing of skin, salivation, cirrhosis, abortion, myocarditis, periphuritis, nephritis, diarrhea, hypogonadism, taste changes, delay in wound healing, skeletal deformity, osteomalacia, hyperkeratosis, anorexia, nausea & vomiting, headache, anemia, degenerative disorders, and arthritis (Abdelhamid, 2000 and 2006).

Therefore, Abdelhamid (2006) focused on the sources and importance of heavy metals in aquatic environments and their toxic effects on fish as well as on humans, and their spread in Egyptian water, with different images (Nile, lakes, seas) Moreover, between sampling sites related to the content of heavy metals in fish culture tested and in the same tested fish by many researchers. Abdelhamid et al. (2006 a) reported that the concentration of heavy metals varies according to the sampling sites in the
mullet (from both natural fisheries and cultivated desert). Fish farms are exposed to many sources of pollution with heavy metals, whether from its soil (Abdelhamid et al., 1997). Rearing water contaminated with different drainage sources (Abdelhamid and El-Zareef, 1996), agricultural (Abdelhamid and Ibrahim, 2003), industrial (Abdelhamid et al., 2000b and 2013b), and sanitary (Abdelhamid et al., 2014 a, b and c). Physiological responses of fish may be adversely affected by these factors that affecting the life of fish and their production (Abdelhamid et al., 2000a & 2006b, Abdelhamid, 2006 and Abdelhamid et al., 2014 c). From the physiological point of view, Abdelhamid et al. (2006a) found that heavy metals raised number of total white blood cells, as well as the activity of leucocytes in the serum, and differential segregation, while intestinal dissection and skin mucus had low activity in addition to red blood cells, number of platelets, and serum protein were decreased. Hemoglobin and hematocrit values also were declined.

Table 6: Mean* values of heavy metals Pb, Cd, and Hg (mg/ L) in the fish tissues from Mediterranean Sea, Manzalh Lake and the Fish farm at zero time and after soaking in Pomegranate agent at 30 min

<table>
<thead>
<tr>
<th>Place</th>
<th>Treatments</th>
<th>Period</th>
<th>Pb</th>
<th>Cd</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mediterranean Sea</td>
<td></td>
<td>0</td>
<td>0.547±0.148ª</td>
<td>0.036±0.017ª</td>
<td>0.215±0.086ª</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>0.006±0.126ª</td>
<td>0.003±0.015ª</td>
<td>0.090±0.088ª</td>
</tr>
<tr>
<td>Manzalh Lake</td>
<td></td>
<td>0</td>
<td>1.304±0.224ª</td>
<td>0.000±0.000ª</td>
<td>0.000±0.001ª</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>0.059±0.159ª</td>
<td>0.000±0.000</td>
<td>0.000±0.000ª</td>
</tr>
<tr>
<td>Fish Farm</td>
<td></td>
<td>0</td>
<td>0.278±0.197ª</td>
<td>0.196±0.141ª</td>
<td>0.019±0.013ª</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>0.000±0.000ª</td>
<td>0.000±0.000ª</td>
<td>0.000±0.000ª</td>
</tr>
</tbody>
</table>

* Mean values of three replicates samples ± Standard Deviation
a, b and c:Mean with different letters in the same column significantly differ(P≤0.05).

The effect of the treatment agent differed depending on different variables as the effect of the interaction (s). The treatment generally reduced the heavy metals concentration comparing with zero time before treatment. Moreover, prolonged treatment period (20 and 30 min.) generally reduced (P≤0.05) the heavy metals level, particularly in Pb and Cd. Pomegranate reduced Hg level in the fish flesh. Data in Table 7 shows that the removal percentage of heavy metals Pb, Cd, and Hg in the fish
tissues from Mediterranean sea, Manzalh Lake and the fish farm after soaking in Pomegranate agent at 30 min we can note that the pomegranate peel was removed by a large percentage the lead from the fish samples in a general manner, where it was removed completely from the farm fish samples followed by the sea fish samples and then the farm fish samples. Cadmium was also removed completely from farm fish samples and removed by 91% of sea fish samples ,as such pomegranate peel was completely removed Hg from the farm and lake fish samples but its mercury removal rate from sea samples was lower.

On the other hand, the pomegranate peel of the three heavy metals removed from sea fish samples was Pb> Cd > Hg , while the heavy metal removal by pomegranate peel from the lake fish samples was Pb> Hg whilst farm fish samples were not contaminated with Cd , however, we note from the table that soaking fish samples for 30 minutes in pomegranate peel solution led to the decontamination of the three heavy metals from fish samples of farm.

Pomegranate peel (the non-edible portion) contains about three times the total amount of polyphenols, including intensive tannins (Plumb et al., 2002), gallocatechins, prodelphinidins and catechins (Chidambara et al., 2002). Pomegranate peel (internal and external) is about 26-30% of the weight of the fruit and contains many antioxidants. In large quantities, for example, tannins and flavonoids are mainly concentrated in the skin, and contain. 92% of calcium total antioxidants in fruit (Ismail et al., 2012). Therefore, the peel of pomegranate fruits has fewer benefits than its skin (Shark, 2016). Abdulrazik (2016).

Used pomegranate peel to remove lead from drainage water and proved that the pomegranate peel content of the antioxidant is considered to be highly effective in removing toxic mineral substances.

Table 7: The removal percentage of heavy metals Pb, Cd, and Hg in the fish tissues from Mediterranean Sea, Manzalh Lake and the fish farm after soaking in Pomegranate agent at 30 min

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Location</th>
<th>Pb</th>
<th>Cd</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pomegranate</td>
<td>Mediterranean Sea</td>
<td>98.90%</td>
<td>91.67%</td>
<td>58.14%</td>
</tr>
<tr>
<td></td>
<td>Manzalh Lake</td>
<td>95.47%</td>
<td>zero%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Fish farm</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Conclusion

The obtained results revealed that, all tested water and grey mullet (*M. cephalus*) samples collected from different locations were contaminated with higher Cd, Hg and/or Pb than the tolerance levels. Treating fish before cooking with pomegranate peels (as a natural agent) for 20 – 30 minutes reduced the heavy metals' residues in the fishflesh. Consequently, it is possible to recommend the importance of soaking fish before cooking in the water extract of pomegranate peel (1 g pomegranate powder / 100 g fish) for 20 or 30 minutes to reduce the concentration of these dangerous pollutants in fish flesh and make them safe for human consumption. Yet, it still that the prophylactics are better than treating, i.e. treating different drainages before turnoff the waste waters into surface water is a must. Further studies are seriously required to evaluate the role of such natural treating agents for removing the different contaminates agents, which could help in reducing the residues of such contaminants.

Reference


تأثير محاليل مائية من قشر الرمان على بعض العناصر الثقيلة في أسماك البورى

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الملخص العربي:
عينات الماء وأسماك البورى التي تم تجميعها من البحر المتوسط وبحيرة المنزلة ومزرعة سمكية خاصه كانت ملوثة بالكادميوم، الزنبق، وأي الرصاص. تركيزات الرصاص كانت بالقصص تركيز في مياه البحر، بينما أقصى تركيزات للكادميوم والزنبق كانت في مياه المزرعة السمكية. وعموماً مستوى الرصاص في المياه كان أعلى من مستوى كل من الكادميوم والزنبق، على التوالي. كانت هناك فروقاً معروفاً بين المواقع المختبر وكذلكل بين فترات المعالمة بالنسبة لتركيزات العناصر الثلاثة في عينات الأسماك. أظهرت التحاليل الكيميائية أن أسماك البورى احتوت على مستوى من الرصاص، بينما أسماك المزرعة السمكية احتوت أعلى تركيزات للكادميوم. بصفة عامة، كانت أسماك البحر هي الأعلى احتواء للزنبق. خفض قشر الرمان من تركيزات العناصر الثلاثة لحم الأسماك، وأكثر من ذلك، أدت إطالة فترة المعالمة (30 دقيقة) للأسماك إلى خفض مستوى العناصر الثقيلة.

خاصة الرصاص والكادميوم. وبالتالي يمكن التوصية بأهمية نقع الأسماك قبل الطهي في المستخلص المائي لقصر الرمان (1 جم مسحوق قشر رمان/ 100 جم سمك) لمدة (20 أو 30 دقيقة) لخفض تركيز تلك الملوثات الخطرة في لحوم الأسماك وجعلها آمنة للإستهلاك الآدمي.