ACCUMULATION OF ALUMINUM IN BOLTI (*Tilapia nilotica)* FISH

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Abstract: Bolti (*Tilapia nilotica*) Fish fingers were reared in water contained three levels of Al ions for two month. Technological parameters of a life fish as well as gross chemical composition of fish flesh were determined. Soluble protein was fractionated by using Ultrathin-layer isoelectric focusing on polyacrylamide gel (150µm thickness). Al concentration was determined in the main three parts of fish (head, viscera and flesh). Results revealed that Al pollution of fish environment caused a reduction in fish weight and length as well as increase the weight of fish viscera. Consequently, fish stability as food declined. Significant ($p \leq 0.05$) differences were noticed in chemical composition of Bolti fish due to the presence of Al ions in fish environment, where protein content of fish flesh decreased. while both mean ash and lipid content increased. Fish viscera had the significantly ($p \leq 0.05$) highest Al content followed by fish head and the lowest value was detected in fish flesh. The higher the Al concentration in water environment, the upper the Al content in fish parts**.** Isoelectric focusing of fish protein pattern was changed due to Al-pollution. Since, the lower the Al-pollution, the more color intensity of separated protein bands. In the meantime, some protein bands were totally disappeared between pH 7.3 to 8.3 at high Al concentration up 6 ppm in the reared water.

Keywords: Aluminum, Tilapia, Physical parameters, Chemical composition, Isoelectric focusing

Introduction

Fish is an important part of animal protein in Egypt. Bolti (a common local name of Tilapia) fish is popular and favorite kind of fish. It is commercially speared and represent about 40% of the total catch of fish in Egypt (GAFRD, 2000). Tilapia farming is now wide spread in Egypt. The major problem of aquaculture in Egypt is the pollution of fish environment, since the water of agricultural and industrial effluents are usually used as a source of growing media. Therefore, most of farmed fish as well as fish that captured from drains are normally polluted with heavy metals and some organic chemicals, especially, pesticide residues (McCrea & Fischer, 1986 and Wageman, 1989).

Industrial, agricultural and sewage effluent usually involves organic matters, metals and up to a million different pollutants which

discharged directly into natural water. As a result, lakes, rivers and coastal water are transformed into swage depots (Borg, 1987 and Boult *et al*, 1994). Heavy metals could be accumulated from water to higher levels in edible tissues of fish (Atta *et al*, 1997). The uptake of metals by fish depends, mainly, on the type of fish, concentration and mobility of individual metal in the ecosystem (Atta, 1995a , b and Owon *et al*, 1995).

Aluminum is an ion that doesn't get much discussion in reef keeping circles. It has little in the way of positive biological functions. Interestingly, aluminum is present at much higher total concentration in the Mediterranean Sea (0.00008 – 0.02 ppm) (El-Nady & Dowidar ,1997) than in the Pacific Ocean (0.0000016 – 0.00016 ppm) (Orians & Bruland,1986 and Moran *et al*, 1992) or near Antarctica (0.00008 ppm) (Measures and Edmond,1990). Aluminum concentration in some shore areas are also elevated due to the input from rivers (Vink & Measures2001). Based on limited early studies, there is little indication that aluminum is carcinogenic (Leonard & Gerber, 1988 and Bhamra & Costa, 1992). However, it has been suggested that aluminum exposure is a risk factor for the development or acceleration of onset of Alzheimer's disease (AD) in humans (Crapper McLachlan, 1986 and Crapper McLachlan *et al*, 1989).

The precise pathogenic role of aluminum in AD is judged controversial and remains to be defined (Wisniewski & Wen, 1992; Wischik *et al*, 1992 and Edwardson, 1992). The bioavailability and toxicity of aluminum varies with its chemical speciation. In the case of fish, higher polymers are less toxic than monomers and polymers of low relative molecular mass.

Polymerization is a slow process, hence the biological activity of aluminum in water depends not only on aluminum concentration and conditions such as pH, temperature and the presence of complexing ions, but can also depend on the pre-history of the water. The various aluminum species differ in their effects on fish gills, either disturbing the ion balance or interfering with respiration. The toxicity diminishes if the aluminum is inactivated by complexation with organic ligands, fluoride or silicate, or by extensive polymerization to large molecules in the water (Rosseland & Staurnes, 1994). On the other hand, toxicity of Al increases at low pH (Poléo, 1995) and high temperature (Peuranen *et al*, 2003).

In freshwater systems, solubility and toxicity of aluminum depends on the pH of environment. Whereas solubility and the nature of the interaction of aluminum with the surfaces of organisms increase at lower pH (Sparling & Lowe, 1996). At pH 7, aluminum can bind to the

gills of fish, inducing asphyxiation. (Sparling *et al*, 1997). The maximum solubility of aluminum at pH 8.2 in freshwater is about 2.7 ppm which will be precipitated as amorphous aluminum hydroxide at higher concentration (Pankow, 1991).

Considerable evidence indicates that aluminium is neurotoxic to experimental animals. The brain aluminium concentration necessary to achieve LD_{50} in rabbits is about 6 µg aluminium/g dry weight (Crapper McLachlan *et al*, 1989 and McLachlan & Massiah, 1992). The normal brain aluminium concent-ration in healthy rabbits is approximately 1.1 µg/dry weight. It has been suggested that aluminium exposure is a risk factor for the development or acceleration of Alzheimer's disease in humans (Crapper McLachlan, 1986 and Crapper McLachlan *et al*, 1989). The precise pathogenic role of aluminium in Alzheimer's disease is judged controversial and remains to be defined (Wisniewski & Wen, 1992; Wischik *et al*, 1992 and Edwardson, 1992).

The bioavailability and toxicity of aluminium varies with its chemical speciation. In case of fish, higher polymers are less toxic than monomers and polymers of low relative molecular mass. Polymerization is a slow process, hence the biological activity of aluminium in water depends not only on aluminium concentration and conditions such as pH, temperature and the presence of complexing ions, but can also depend on the pre-history of the water. The various aluminium species differ in their effects on fish gills, either disturbing the ion balance or interfering with respiration. Thus, many articles in the literature dealt with aluminium and other metals content of aqua ecosystem in order to establish their normal concentration range and evaluate their role in fish as a part of food chain (Baker & Schofield1982; Berg & Burns1985; Bache ,1986; Bird *et al,* 1990; Sparling & Lowe, 1996; Sparling *et al*, 1997; Sacan & Balcioglu, 2001and Randy, 2003).

This work was designed to throw some light on the bioaccumulation of aluminum in Bolti fish (*Tilapia nilotica*) parts (head, viscera and flesh) grown in aquarium contained polluted water with aluminum ions at three levels (3, 6 and 9 ppm).

Materials and Methods

2.1. Materials

Bolti fish (*Tilapia nilotica*) fingers were supplied from fish farm of Arab Fisheries Company, Berseeq. Abou-Hommos, Behera governorate, Egypt. Twenty five specimens were placed in a glass tank (70×35×35cm) containing 80 liters of dechlorinated and aerated tap water under controlled conditions (24 -26°C, pH 7.1-7.5) to

avoid high toxicity of Al at low pH and acidic medium (Peuranen *et al*, 2003). Fish were acclimatized for one week prior to the test initiation under laboratory conditions (until the specimens weight reach about 25-30 g). Aluminum ions was introduced to water at three levels 3ppm (Treatment I); 6ppm (treatment II) and 9ppm (treatment III) in the form of $Al_2(SO_4)_3$. A control was carried out by growing fish in dechlorinated and aerated tap water under the same conditions. The period of experiment was two month.

Pre-coated ultra-thin layer polyacrylamid gel UTL-PAG 150μm thickness (pH range 4-7), Marker protein 9 and Coomassie Brilliant Blue R_{250} were purchased from Serva (Heidelberg, Germany). Electrode strips (0.5 cm thickness) cellulose paper MN866 were obtained from Macherey & Nagel Co. (Duren, Germany). Other chemicals used in this study are analytical grades.

2.2. Methods

2.2.1. Physical analysis

At the end of experiment, 10 specimens were washed twice with distilled water. Length of fish body and fish head were taken by using an accurate metal ruler. Total weight in gram and length in cm of fish were recorded, then each specimen was dissected to separate head, viscera and flesh. The individual part was collected and weighed (in gram).

2.2.2. Fish preparation for chemical analysis

The separated flesh fragments were collected and homogenized using stainless steel hand grinder. Fish head and viscera were also homogenized , individually, following the same procedure used for flesh. The homogenized samples were kept in tightly sealed polyethylene bags for further analysis.

2.2.3. Chemical analysis for fish parts

Moisture, crude protein (N×6.25) , crude lipid and ash content of fish parts were determined according to AOAC (1995). Aluminum content of fish parts was estimated following the wet digestion (Kopito, 1970) using a nitric: perchloric acid mixture (4:1 v/v). The digest was quantitatively transferred to a 25-ml volumetric flask with twice-distilled water, adjusted to pH 2 with nitric acid and the volume was made up to the mark. Aluminum was measured using Atomic Absorption Spectroscopy (Laboratory Instrumentation Model AAC105). Aluminum concentration was expressed as μg metal per gram dry weight fish tissue.

2.2.3. Isoelectric focusing of fish protein

Fish protein was recovered using the method described by Atia & Radola (1986) by pressing the fish flesh with hand presser. The yield juice was centrifuged at 4000 rpm for15 min, then the lipid layer was skimmed off. The fat-free fish protein was subjected to ultrathin-layer focusing onto 150µm thickness polyacrylamide gel (pH 5-8) using a Pharmacia flat-bed chmber, type FRE 3000 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) at 4°C through circulating bath system (Colora type WK 3 from Colora Masstechnik GmbH, Wurtt., Germany). Focusing conditions was: 750 volt for 30 min followed by 1000volt for 40 min, then 1200 volt for 30 min and finally 1500 volt for 3 min. The total vh was 1691~1700. Fixing, staining and destaining of the focused gel were carried out as described by Radola (1980). Three runs for each extract were performed to insure repeatability.

2.2.4. Statistical analysis

Results represent means and standard deviation (M±SD) for three replicates calculated by Microsoft Excel software. Statistical analysis were determined by using Duncan's multiple rang test at p<0.05 by Irristate computer program version 92-1

Results and Discussion

3.1. Physical parameters

Data in Table (1) showed that body and head length as well as body weight of fish reared in polluted water with Al till 6 ppm (treatment II) were not significantly ($p \leq 0.05$) different than that of control. While fish grown in water contained 9 ppm Al (treatment III) had significantly ($p \leq 0.05$) lower body length and weight than that of control ones. Weight of fish viscera was the most impressed parts by Al pollution, where the weight of viscera of fish grown in high polluted water with Al was significantly ($p \leq 0.05$) higher heavier than that of control one. As the higher the Al pollution of fish environment, the heaver the fish viscera. It is well known that ratio of body weight to body length ($W/L^3 \times 100$) is usually taken to determined the edible part of fish or fish stability as food. Results of Table (1) revealed that ratio of body weight to body length ($W/L^3 \times 100$), influenced by the concentration of Al ions in reared water. Since, the significantly ($p \leq 0.05$) highest ratio (41.70) was found in fish reared in water contained 9 ppm Al (treatment III) followed by fish reared in water contained 6ppm Al (treatment II). Conversely, the significantly ($p \leq 0.05$) lowest ratio was detected in control fish samples.

In conclusion, Al pollution of fish environment caused a reduction in fish weight and length as well as increase the weight of fish viscera.

The low weight of whole fish body with weighty fish viscera means that the waste of polluted fish is higher than that of non-polluted one. Consequently, augment of $W/L^3 \times 100$ ratio decreases the fish stability as food.

Table (1): Technological Parameters of Bolti Flesh (*Tilapia nilotica*) Fish Fingers After Two Month Rearing in a Polluted Water with Aluminum at Different Levels (3, 6 and 9 ppm)

Fish Parameters	Control	Treatment I	Treatment II	Treatment III
Length of body (cm)	5.4 ± 0.3^a	5.4 ± 0.3^a	5.3 ± 0.4^a	5.0 ± 0.3^b
Length of head (cm)	1.5 ± 0.3^a	1.4 ± 0.3^a	1.5 ± 0.3^a	1.4 ± 0.2^b
Weight of body (g)	$55..9 \pm 3.2^a$	$55..5 \pm 5.3^a$	54.6 ± 3.5^a	50.6 ± 5.2^b
Weight of head (g)	14.1 ± 1.3^c	14.5 ± 1.3^b	14.5 ± 2.1^b	15.2 ± 3.0^a
Weight of Viscera (g)	4.8 ± 1.2^c	5.1 ± 1.5^b	5.2 ± 1.9^b	5.6 ± 2.1^a
Head length to body length	27.0 %	26.9 %	27.5 %	27.9 %
Head weight to body weight	25.1 %	26.1 %	26.5 %	30.0 %
Viscera weight / body weight	8.6 %	9.2 %	9.5 %	11.0%
W/L^3 X 100	35.5 ± 1.3^c	36.3 ± 1.2^{cb}	37.3 ± 1.4^b	41.7 ± 1.4^a

Treatment I = (3ppm)   Treatment II= (6ppm)

Treatment III= (9ppm)

M±SD   = Means and standard deviation where n = 15

In a raw, means having the same superscript letters are not significantly different at 0.05% level

3.2. Gross chemical composition of fish parts

Data in Table (2) and Fig. (1) revealed that moisture, crude protein, crude lipid and ash contents (based on wet weight basis) of Bolti fish (*Tilapia nilotica*) flesh were 77.1%, 20.0%, 1.5% and 1.2%, respectively. Whereas protein, lipid and ash contents (based on dry weight basis) were 87.2%, 6.7% and 5.2% respectively. These results are in agreement with those reported by Fouda *et al*, (1984), Noaman *et al*, (1993), Al-Kahtani *et al*, (1996) and Keshk (2004).

The same data display that moisture content of Tilapia's flesh

(*Tilapia nilotica*) fish was not significantly (*p≤0.05*) changed due to Al pollution in fish environment. While protein content of flesh removed from fish reared in water contained 3 ppm Al (treatment I) was significantly (*p≤0.05*) higher than that of fish flesh obtained from fish grown in water contained 6 and 9 ppm Al (Treatments II and III). However, crude lipid and ash content of fish flesh extracted from fish reared in water contained 3 ppm Al (treatment I) were significantly (*p≤0.05*) higher than those found in control one. These results agree with Atta *et al* (1997) who reported that significant differences could be noticed in chemical composition of Tilapia fish due to the presence of heavy metal in fish environment.

Table(2): Gross Chemical Composition of Bolti Flesh (*Tilapia nilotica*) Fish Fingers After Two Month Rearing in a Polluted Water with Aluminum at Different Levels (3, 6 and 9 ppm)

	Control	Treatment I	Treatment II	Treatment III
	(g/100g wet weight basis)			
Moisture	77.1 ± 1.2^a	77.0 ± 1.3^a	$77.6 1\pm 1.0^a$	77.2 ± 1.2^a
Total Solids	22.9 ± 1.2^a	23.0 ± 1.3^a	23.39 ± 1.0^a	22.8 ± 1.2^a
Protein	20.0 ± 0.8^a	20.1 ± 0.9^a	19.15 ± 1.0^b	19.3 ± 1.0^b
Crude lipid	1.5 ± 0.2^c	1.6 ± 0.2^b	1.75 ± 0.2^a	1.8 ± 0.2^a
Ash	1.2 ± 0.2^c	1.4 ± 0.2^b	1.67 ± 0.2^a	1.8 ± 0.2^a
	(g/100g dry weight basis)			
Protein	87.2 ± 1.6^a	86.8 ± 1.6^a	85.4 ± 1.6^b	85.6 ± 1.6^b
Crude lipid	6.7 ± 0.9^b	7.0 ± 1.0^b	7.8 ± 0.9^a	7.9 ± 1.1^a
Ash	5.2 ± 0.8^c	5.8 ± 0.8^b	7.5 ± 1.0^a	7.9 ± 1.1^a

Treatment I   = Treatment no I   (3ppm)

Treatment II = Treatment no II   (6ppm)

Treatment III = Treatment no III (9ppm)

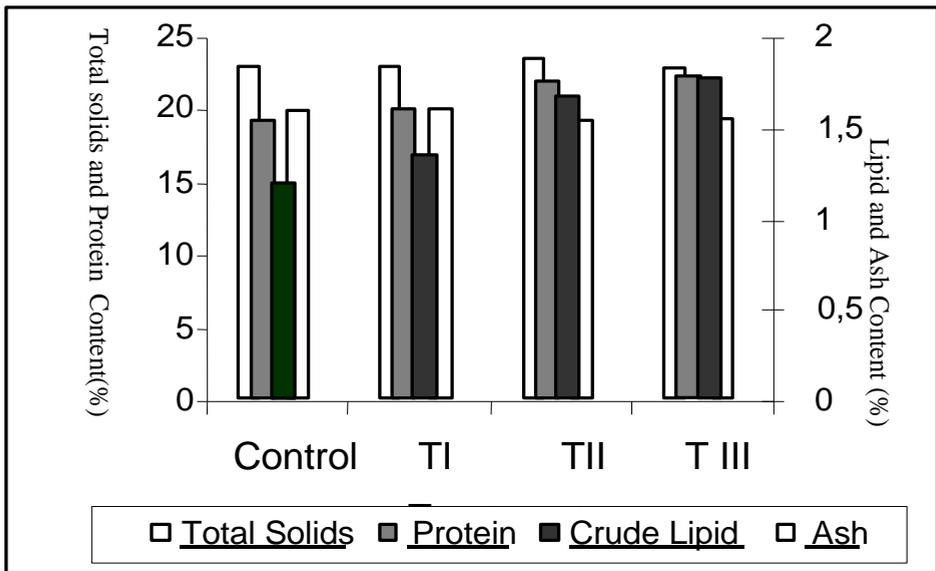M±SD   = Means and standard deviation of triplicate determination

Fig.(1): Total solids, protein, ash and crude lipid content of Bolti (*Tilapia nilotica*) fish fingers grown in water contained different levels of Al (3, 6 and 9 ppm)

TI= Fish water environment contained 3 ppm Al ions

TII= Fish water environment contained 6 ppm Al ions

TII= Fish water environment contained 9 ppm Al ions

3.3. Aluminum concentration of fish parts

Table (3) revealed that distribution of Al ions in Bolti (*Tilapia nilotica*) fish was not depend on the concentration of Al in water environment only, but also on the nature and type of fish part. Whereas fish viscera had the significantly ($p \leq 0.05$) highest Al concentration (110.3, 1958.4, 2541.2 and 3181.3 mg/Kg) among the other fish parts followed by fish head

(28.1, 50.1, 71.1 and 89.9 mg/Kg). While the significantly ($p \leq 0.05$) lowest Al concentration (18.1, 36.3, 70.9and 72.7 mg/Kg) was found in fish flesh. The same data displayed that as the concentration of Al ions in fish water environment increase, Al level in fish parts was also significantly ($p \leq 0.05$) raised. Theses results are in full accordance with those reported by Lacroix *et al*, (1993); Atta (1995a,b); Atta *et al* (1997) and Kroglund and Finstad

(2003). They pointed out that fish viscera including the internal organs is the most polluted parts with metals flowed by head including gills.

3.4. Isoelectric focusing of fish flesh on ultrathin-layer polyacrylamid gel

As shown in Fig (2) protein pattern of extracted from control fish was different than those of fish grown in Al-polluted water. For instance protein pattern toke out control fish had the highest protein concentration (regarding to the color intensity of protein bands on the separating gel) followed by protein pattern of fish reared in water contained 3 ppm Al (treatment I) which was more intense than that one of fish grown in water contained 6 ppm Al (treatment II). While protein pattern of soluble protein removed from fish reared in water contained 9ppm Al (treatment III) had the weakness color intensity among the other protein patterns of other studied fish. Moreover, fish protein toke out fish reared in water contained 6 ppm Al ions (treatment II) had some weak protein bands between pH 7.3 to 8.3 which totally disappeared in treatment III comparing with control one. It is clear that as the concentration of Al ions increased in the water environment of fish, the color intensity of protein pattern was more faint This means that some proteins could be broken down due to the presence of Al ions in the aqua system of fish. These results were confirmed by fish protein content whereas the protein concentration of control fish flesh was significantly ($p<0.05$) higher than those found in fish grown in Al-polluted water, especially, at high concentration level (treatment III).

Table(3): Aluminum Concentration (as mg Al/kg fish dry weight basis) of Bolti (*Tilapia nilotica*) Fish Fingers After Two Month Rearing in a Polluted Water with Aluminum at Different Levels (3, 6 and 9 ppm)

Fish parts	Control	Treatment I	Treatment II	Treatment III
Head	28.1 ± 0.3^d	50.1 ± 0.3^c	71.1 ± 0.4^b	89.9 ± 0.3^a
Viscera	110.3 ± 0.3^d	1958.4 ± 0.2^c	2541.2 ± 0.3^b	3181.3 ± 0.3^a
Flesh	18.1 ± 3.2^d	36.3 ± 5.3^c	70.9 ± 3.5^b	72.7 ± 5.2^a

Treatment I   =   (3ppm)
Treatment II   =   (6ppm)
Treatment III =   (9ppm)
M±SD   = Means and standard deviation of triplicates analysis
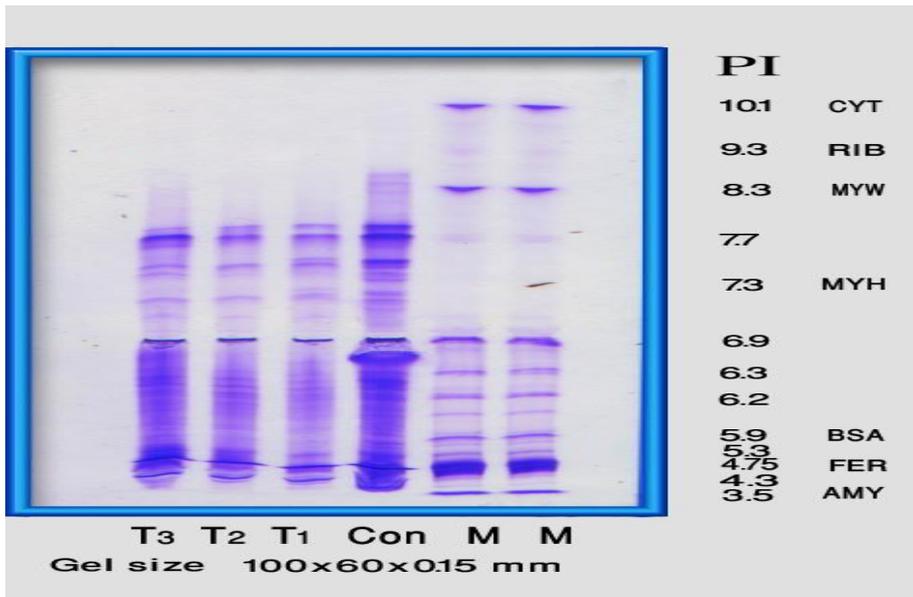In a raw, means having the same superscript letters are not significantly different at 0.05% level

Fig.(2): Isoelectric focusing of soluble protein extracted from Bolti (*Tilapia nilotica*) fish fingers reared for 3 months in Al polluted water at three different levels (3, 6 and 9 ppm) on Ultrathin-layer polyacrylamid gel (150µm) containing carrier ampholyte pH 4-7 T1 = (3ppm) T2 = (6ppm) T3 = (9ppm)   M = Marker proteins

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تراكم الألمنيوم في السمك البلطي

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قسم تكنولوجيا اللحوم و الأسماك- معهد تكنولوجيا الأغذية - مركز البحوث الزراعية- الجيزة

اهتم البحث بقدير الصفات التكنولوجية Technological properties والكيماوية Gross chemical composition وكذلك تركيز معدن الألمنيوم في أصباعيات سمك البلطي Tilapia fingers المنمي لمدة شهرين في مياه تحتوي علي ثلاث تركيزات مختلفة من الألمنيوم. كما تم فصل البروتينات الذائبة في لحم السمكة باستخدام الفصل الكهربائي علي طبقة رقيقة من الجيل Ultrathin-layer isoelectric focusing لمعرفة مدي الاختلافات في البروتينات المفصولة نتيجة نمو السمك في البيئة الملوثة بمعدن الألمنيوم. و قد بينت النتائج المتحصل عليها أن وزن و طول السمكة يقل مع زيادة وزن الأحشاء الداخلية لها بزيادة تركيز الألمنيوم في البيئة المائية للسمك. وبالتالي يقل كمية الجزء المأكول من السمكة The food stability of fish as food decreased. كذلك يتأثر التركيب الكيماوي للسمكة حيث تقل النسبة المئوية للبروتين في لحم السمكة مع زيادة النسبة المئوية لكل من الرماد و اللبيبيدات و خاصة في الأحشاء. هذا وقد لوحظ أن تركيز الألمنيوم في الأجزاء المختلفة (الرأس- الأحشاء – اللحم) يزيد بزيادة تركيز المعدن في البيئة المائية التي ينمو بها السمك. و تعتبر الأحشاء أكثر الأجزاء تلوثا بالمعدن يليها الرأس. أما لحم السمكة فكانت أقل الأجزاء تأثرا بالتلوث حيث احتوت علي أقل تركيز من الألمنيوم مقارنة بالأجزاء الأخرى. أظهر الفصل الكهربائي للبروتينات الذائبة أن التلوث بالألمونيوم له تأثير واضح في تركيز البروتينات المفصولة وأن زيادة التلوث يؤدي إلي خفض تركيز البروتينات المفصولة حتى أن بعض البروتينات القلوية (pH 7.3 to 8.3) قد اختفت تماما.