

DETERMINATION OF P, Ca, Zn, Cd AND Pb CONCENTRATIONS IN MUSCLE, GILLS, LIVER, GONADS AND SKELETON OF TWO NATURAL POPULATIONS OF *ATHERINA LAGUNAE* IN NORTH LAKE OF TUNIS, (TUNISIA)

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ملخص

تحديد ، تركيزات الزنك ، الكاديوم ، الرصاص ، الفسفور ، الكالسيوم في العضلات والخياشيم والكبد والغدد التناسلية والهيكل العظمي لدى مجموعتين من الأثرنا *Atherina lagunae* في بحيرة تونس الشمالية : في هذه الدراسة ، نبحث عن تركيزات الزنك ، الكاديوم ، الرصاص ، الفسفور ، الكالسيوم في العضلات والخياشيم والكبد والغدد التناسلية والهيكل العظمي لدى مجموعتين من الأثرنا *Atherina lagunae* العادية والمشوهة ، فضلا عن التراكم الأحيائي لهذه العناصر في الماء والرواسب في بحيرة تونس الشمالية. تم إجراء تحليل الكالسيوم بواسطة الامتصاص الذري مع لهب. متوسط تركيزات الكالسيوم في الأنسجة المختلفة تظهر أعلى القيم لدى الأثرنا العادية إلا في الغدد التناسلية حيث يفوق بكثير متوسط تركيز الكالسيوم في الأثرنا المشوهة ($p < 0,05$) ، و في العمود الفقري تركيزات الكالسيوم مماثلة في كلا المجموعتين. وتم تحديد الزنك والكاديوم والرصاص والفسفور بواسطة (ICP-AES) spectrométrie d'émission plasma à couplage inductif. وكانت تركيزات الزنك لدى الأثرنا *Atherina lagunae* في شمال بحيرة تونس عالية جدا بالمقارنة مع دراسات أخرى في بحيرات أخرى. ووجدت أعلى التركيزات لدى الأثرنا المشوهة. يبين التحليل أن القيم العليا لتركيز الفسفور في مختلف الأنسجة موجود لدى الأثرنا العادية. وكان المعدل المحتمل للكاديوم دون الحد الأدنى للكشف في مختلف الأنسجة التي تم تحليلها ، في المياه والرسوبيات. الكلمات المفتاحية: المعادن الثقيلة والفسفور والكالسيوم ، وتشوه العمود الفقري ، شمال بحيرة تونس ، *Atherina lagunae*.

RESUME

Détermination des concentrations de P, Ca, Zn, Cd et Pb dans les muscles, les branchies, le foie, les gonades et le squelette de deux populations naturelles de *Atherina lagunae* dans le lac nord de Tunis, (Tunisie) : Dans cette étude, les concentrations du zinc (Zn), du cadmium (Cd), du plomb (Pb), du phosphore (P) et du calcium (Ca) dans les muscles, les branchies, le foie, les gonades et le squelette chez deux populations naturelles d'athérine lagunaire (*Atherina lagunae*, téléostéens, Atherinidae) normale et déformées, ainsi que la bioaccumulation de ces éléments dans l'eau et les sédiments dans le lac Nord de Tunis ont été étudiée. L'analyse de calcium a été réalisée avec la spectrométrie d'absorption atomique à flamme. Les concentrations moyennes de Ca dans les différents tissus analysés montrent des valeurs plus élevées chez la population saine d'athérines, sauf dans les gonades, où la concentration moyenne de calcium chez les athérines déformées est nettement supérieur à celle des athérines normale ($p < 0,05$). La concentration de Ca au niveau de la colonne vertébrale est la même dans les deux populations. Le zinc, le cadmium, le plomb et le phosphore ont été déterminés par spectrométrie d'émission plasma à couplage inductif (ICP-AES). Les concentrations de Zn chez *lagunae Atherina* du lac nord de Tunis ont été très élevées par rapport à d'autres études dans les lagunes d'autres. Les plus fortes concentrations ont été trouvées chez les athérines déformées. Les différences sont significatives pour tous les tissus étudiés ($p < 0,05$). La concentration moyenne de P dans les différents tissus analysés montre que les valeurs les plus élevées ont été détectées dans la population saine de *Atherina lagunae*. Le taux potentiels de Cd a été en-dessous de la limite de détection dans les différents organes analysés, dans l'eau et dans le sédiment.

Mots-clés: métaux lourds, phosphore, calcium, déformation rachidienne, Lac Nord de Tunis, *lagunae Atherina*.

ABSTRACT

In this study, zinc (Zn), cadmium (Cd), lead (Pb), phosphorus (P) and calcium (Ca) concentrations in muscle, gills, liver, gonads and skeleton of two natural populations of sand smelt *Atherina lagunae* (Teleostean, Atherinidae) normal and deformed, as well as bioaccumulation of these elements from the water and the sediment in the North Tunis Lake were investigated. The analysis of calcium was performed with flame atomic absorption spectrometry. The average concentrations of Ca in the different tissues analyzed show higher values in healthy atherines except in gonads where the average concentration of Ca in deformed atherines significantly

exceeds that in normal atherines ($p < 0.05$) and the Ca spine concentrations were similar in the two populations. Zinc, cadmium, lead and phosphorus were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). The Zn concentrations of *Atherina lagunae* in North Tunis Lake were very high compared to other studies in other lagoons. The highest concentrations were found in deformed atherines. The differences are significant for all tissues studied ($p < 0.05$). The average concentration of P in different tissues analyzed shows that the highest values were detected in the normal population of *Atherina lagunae*. The potential rate of Cd was below the limit detection in the different organs analyzed, in water and sediment.

Keywords: heavy metal, phosphorus, calcium, spinal deformation, Tunis North Lake, *Atherina lagunae*.

INTRODUCTION:

The Tunis North Lake, environment receiving domestic and industrial discharge from Tunis City and neighbouring towns, has experienced many dystrophies crises. From 1929 to 1980, the Tunis North Lake has been progressively deteriorated by enrichment of its funds by organic matter, the extinction of animal and marine plant and proliferation of Pollution indicator green algae *Ulva*.

The Tunis North Lake cleansing began in 1985 and lasted three years (Pergnent and Ben Maiz, 2000). The main achievements are:

- The deepening of the lake bottom, particularly in the north side, with a dredging volume of about 10,000,000 m³ of solid material.
- The dredging of the channel Kheireddine, for a maximum exchange with the sea.
- The introduction of hydraulic doors (locks) at the channel Kheireddine (opening and closing triggered by the tide), allowing control of water exchange with the sea water entering sea water from the Nord at high tide and drainage water lagoon to the south at low tide.
- The construction of a central dam 8.2 km long in the middle of the lake, connecting the entrance to the Chekli Island and separating the lake into two parts to create a cyclonic circulation in the water.
- The filling of nearly 500 ha of land, bringing the surface of the water to 2,500 ha.
- Changing the line of banks (as a straight profile) to avoid stagnation of water, leading to 24 km periphery.
- Establishing a system of renewal of waters was upon completion of remediation to have a better flow of water into the lake, with a daily input of about 1.6 million m³ seawater and a time to stay relatively small (19 to 27 days).
- The drainage works have resulted in improved water quality and hydrodynamic conditions of the environment was responsible for the improvement of biological diversity (over 35 species of fish in 1995 against 24 in 1986 and 38 plant species in 1995 against a dozen in the early 80s).

The analysis of water carried out in different sites in the North Lake of Tunis showed the detection of many potentially harmful species such as *Pseudonitzschia* spp., *Dinophysis* spp., *Alexandrium* spp., *Prorocentrum* spp., *Gyrodinium* spp. and *Coolia*

monotis. The potentially harmful phytoplankton group abundance accounted for 42% of all the phytoplankton species detected. The composition of potentially harmful phytoplankton genera and their succession were related to the available nutrients and other environmental factors. Furthermore, there was a highly positive correlation between the occurrence of potentially harmful species and the two following factors: water salinity and temperature (Turki *et al.*, 2008).

However in 2004, a high rate of spine deformities has been reported affecting fish in the Tunis North Lake such as *Atherina lagunae* (Ayed *et al.*, 2008, 2009), *Diplodus*, *Sparus*, *Dicentrarchus* and *Syngnathus* (unpublished results).

- The effects of environmental disturbances studies of physic-chemical parameters on the spine showed that low temperatures and oxygen deficiency can lead to deformities in *Salmo gairdneri* (Tomasik *et al.*, 1982). High temperatures may also have similar effects in *Cyprinus carpio* (Mis *et al.*, 1995).

Toxic substances such as heavy metals considered among the most dangerous contaminants, can cause spine deformities (Beattie and Pascoe, 1978; Mis *et al.*, 1997).

- Lead can be introduced into the aquatic environment through soil erosion, as atmospheric dust, by oil combustion, by both domestic and industrial landfills and by precipitation (Harte *et al.*, 1991). According to Moriarty (1990), this metal is ubiquitous in the aquatic ecosystem. It is bioaccumulative. It is present as inorganic or organic element. Seymore *et al.* (1995) showed that lead can be metabolized with calcium and subsequently accumulates in bone tissue. It also accumulates in muscle, bone, kidney, gills and liver (Nussey *et al.*, 2000). The toxicity of Lead depends on the fish age, pH and water hardness (Nussey *et al.*, 2000).

- Zinc is a trace element essential for fish metabolism. Several studies have examined both deficiencies and excesses of Zn. The toxic concentrations of Zn vary among aquatic organisms, the exposure time and environmental conditions (Jackson *et al.*, 2005). Zinc accumulates mainly in the gills, intestines and liver.

Cadmium is known for its toxic effects in fish. It causes lesions in the testes and ovaries (Sangalang and O'Halloran, 1973), reducing the consumption of oxygen by the gills. Pathological changes of kidney

and intestinal tissues and lesions and skeletal deformities (Bengtsson *et al.* 1985; Mehrle *et al.*, 1982; Olsson *et al.*, 1999; Teraoka *et al.*, 2002; Cheng *et al.*, 2000).

- The axial skeletal deformities have been linked to dysfunction of bone metabolism in larvae of *Lates calcarifer* (Fraser *et al.*, 2004) and also the muscle degeneration in *Danio rerio* (Gerhard *et al.*, 2002).

- Phosphorus, constituting cell membrane and essential in the phosphorylation reactions plays a major role in the development of bone tissue. The phosphorus absorption by gills cannot meet the phosphorus requirements. Deficiency of this element causes a growth decrease, bone demineralization and skeletal deformities (Kaushik, 2005).

Calcium, such as the phosphorus, plays a - vital role in bone mineralization

In this work, the presence of P, Ca, Zn, Cd and Pb were measured in various organs of *A. lagunae* in both normal and deformed individuals, in water and sediment from North Tunis Lake using several analytical techniques.

MATERIALS AND METHODS:

1. Study area and sampling:

The atherines used in this study were captured from October 2004 to August 2005 in the Northwest of the Tunis North Lake (36°50 N – 10°12 E). The fish were first wrapped into polyethylene plastic, put into an isolated container, and brought to the Biology Laboratory of Tunis Sciences Faculty. After biometric measurements (Table I), the fishes were immediately frozen and stored at -80 °C until dissection. Caught fish were counted. Since spinal deformities were visible on the fish body immediately upon catching, the percentage of deformed fish was calculated. A fish showing a representative deformity were radiographed using a medical X-ray system and the radiographs were used for an examination of the skeleton. A sample of normal fish, based on external morphology, was also radiographed in order to validate the results of the macroscopic evaluation. A total of 300 fish were analyzed, including 150 with spine abnormalities (kyphosis, lordosis and scoliosis) and 150 normal.

Table I Biometric measurements

Size classe (mm)	Numbers of fish	
	Deformed atherine	Healthy atherine
25-30	68	68
30-35	37	37
35-40	25	25
40-45	15	15
45-50	5	5
Total	150	150

2. Sample preparation

To minimize contamination, all the materials used in the experiments were previously washed in ultra pure water, and a stainless steel knife was used to cut the tissues. Before analysis the fish were thawed and a 0.5 g sample was taken from each tissue (muscle, gill, gonads, skeletal and liver). All tissue samples were transferred into 100 ml Teflon beakers. Thereafter, 10 ml ultrapure concentrated nitric acid was added slowly to the sample. The Teflon beaker was covered with a watch glass, and heated at 200°C on a hot plate for 3 h, until the solution evaporate slowly to near dryness. Two millilitres of 1 N HNO₃ was added to the residue and the solution was evaporated again on the hot plate. By repeating the additional digestion twice, all organic materials in each sample were completely digested. After cooling, 2.5 ml of 1 N HNO₃ was added to digested residue

and was transferred to 25 ml volumetric flasks, then diluted to level with deionised water. Before analysis, the samples were filtered through a 0.45 ml nitrocellulose membrane filter. Sample blanks were prepared in the laboratory in a similar manner to the field samples (Alam *et al.*, 2002). Metal contents were expressed as µg.g⁻¹ wet weight for tissues.

3. Chemical analysis

All samples analyses were repeated three times for Cd, P, Pb and Zn by ICP-AES Horiba Jobin Yvon. Standard solutions were prepared from stock solutions (Merck, multi element standard). The accuracy and precision of our results were checked by analyzed certified reference material (CRM, Dorm-2, Dorm-3). The results showed good agreement between the certified and the analytical values (Table II), the recovery of elements being partially complete

for most of them. The absorption wavelengths for Cd, Zn, Pb, and P were 226.502; 213.858; 220.353 and 214.914 nm, respectively.

Calcium was determined by flame atomic absorption with a detection limit of 0.1713 mg/L (Analytik Jena Nava 400).

4. Statistical analyses

Each reported result was the average value of the three analyses. The results were offered as means \pm SEM. A logarithmic transformation was done on the data to improve normality. To test the differences between the concentrations in tissues of fish, one way ANOVA was performed. Post hoc test (Duncan) was applied to determine statistically significant differences following ANOVA. Possibilities less than 0.05 were considered statistically significant ($p < 0.05$). All statistical calculations were performed with SPSS 13.0 for Windows.

RESULTS

1 Incidence of deformities

In the fish population studied, the global incidence of malformations was high. The proportion of kyphosis (Ki), lordosis (Li) and scoliosis (Si) were respectively 9.75% and 8.78%, and 2% of total captured fish. The rate of deformities decreased with age (e.g. 64.94% for size class 25-30 mm and $< 5\%$ for size class 50-55 mm). Kyphosis being the main deformity scored in this way (70% of the observed deformities) (Ayed *et al.*, 2008). External scoliosis had the low incidence level. Among captured population a sample of 300 fish were analyzed (150 normal and 150 deformed fish).

2 Fish

2.1 Calcium

Ca was detected in all muscle samples (deformed fish and healthy). The average concentration is (11.51 ± 0.84) mg/g dry weight in the deformed atherines and (16.55 ± 0.19) mg/g dry weight in healthy atherines. Both samples were analyzed three times. The standard variation respectively in specimens deformed and healthy is of 7.53% and 1.2%. The difference between concentrations was significant ($p < 0.05$). Ca was also detected in the 300 livers tested. The average concentration respectively in the deformed and healthy fish is respectively 5.61 ± 0.86 mg/g dry weight and 7.08 ± 0.4 mg/g dry weight. The standard variation in the deformed is 15.35% and among healthy is 5.72%. Statistical analysis revealed an insignificant difference. The analysis of the skeleton showed a slight non-significant difference between the healthy and deformed samples (56.59 ± 4.73) mg/g dry weight vs. 55.64 ± 1.62 mg/g dry weight). Ca was detected in all samples of gills with average concentrations of 50.401 ± 6.738 mg/g dry

weight in deformed atherines and 62.085 ± 1.939 mg/g dry weight in normal atherines. The standard variations are respectively in the deformed and healthy 13.37% and 3.14%. The highest concentration of Ca measured in the gonads was found in the deformed unlike other organs analyzed (3.59 ± 0.17) mg/g dry weight vs. 2.40 ± 0.19 mg/g dry weight). The difference is statistically significant ($p < 0.005$).

2.2 Zinc

Zn was detected in all tissues analyzed both in healthy and deformed fish. The highest concentrations were found in deformed atherines. The differences are significant for all tissues studied ($p < 0.05$). The maximum value was found in the vertebral column of deformed specimens (309.7 ± 0.76) μ g/g dry weight). In muscle, the average concentration was 142.30 ± 1.87 μ g/g in deformed and 116.59 ± 9.09 μ g/g dry weight in healthy. In the gills, the concentrations are similar in both samples (267.35 ± 0.6) for normal and 295.98 ± 13.06 μ g/g dry weights in deformed) and the differences are not significant. Zn was also detected in all livers analyzed. The average concentrations are respectively in deformed and normal atherines 209.28 ± 10.26 μ g/g and 37.73 ± 3.67 μ g/g dry weights. The difference is highly significant ($p < 0.01$). The highest concentration was 216.53 μ g/g in deformed atherines and 40.33 μ g/g in the normal fish. In the gonads, the concentrations of Zn in deformed specimens were found to be higher than in healthy ones (180.93 ± 10.72) vs 35.97 ± 0.32 μ g/g dry weight) ($p < 0.01$).

2.3 Phosphorus

Phosphorus was detected in all organs analyzed in both healthy and deformed atherines. The differences were not significant in muscles, gills and skeletons. In liver, concentrations in normal atherines were twice time of the deformed fish (14.83) mg/g dry weight vs. 7.25 mg/g). The highest values were detected in the skeleton (53.25) mg/g dry weight in normal vs. 50.09 mg/g dry weight in deformed). The lowest values are noted in the gonads (8.07) mg/g dry weight vs. 5.59 mg/g dry weight). Statistical analysis showed significant differences ($p < 0.05$).

2.4 Cadmium and lead

Cadmium and lead were below the limit detection in the different organs analyzed (liver, gills, muscles, gonads and skeleton), the limit of detection (LOD) is respectively 33.1 ng/l and 272 ng/l. All samples are analyzed three times, but the concentrations of these two elements are always below the detection limits.

3 Water

The potential rate of Pb, Cd and Zn were below the limit of detection. LOD (Pb) = 11 μ g/l; LOD (Cd) =

Table II. Comparison of heavy metal concentration in fish with value taken from open literature, in $\mu\text{g/g}$ dry weight).

Sample area	species	Cd	Pb	Zn	references
Gulf of Gabes (Tunisia) Mediterranean Sea	<i>Zosterisessor ophiocephalus</i> (in liver)	S ₁ N 2.49 ± 0.48 D 7.57 ± 1.09 S N 0.020 ± 0.004 D 0.078 ± 0.017		S ₁ N 22.03 ± 2.44 D 58.93 ± 8.42 S N 6.56 ± 1.38 D 14.75 ± 1.73	Messaoudi <i>et al.</i> , (2008)
Ghar El Melh Lagoon (Tunisia)	<i>Mugil cephalus</i>	Liver 0.678 Muscle 0.022	Liver 0.548 Muscle 0.422		Chouba <i>et al.</i> , (2007)
Gulf of Gabes (Tunisia) Mediterranean Sea	<i>Solea vulgaris</i>	Liver S _a 1.35 ± 0.12 S _b 0.10 ± 0.01 Gill S _a 1.99 ± 0.15 S _b 0.12 ± 0.02			Barhoumi <i>et al.</i> , (2009)
Gulf of Gabes (Tunisia) Mediterranean Sea	<i>Zosterisessor ophiocephalus</i>	Liver S _a 2.59 ± 0.16 S _b 0.18 ± 0.02 Gill S _a 0.80 ± 0.06 S _b 0.16 ± 0.02			Barhoumi <i>et al.</i> , (2009)
Gulf of Gabes (Tunisia) Mediterranean Sea	<i>Salaria basilisca</i>	Liver S _a 4.81 ± 0.13 S _b 0.24 ± 0.02 Gill S _a 2.79 ± 0.10 S _b 0.12 ± 0.01			Barhoumi <i>et al.</i> , (2009)
Southern Atlantic coast of Spain (Bacuta)	<i>Solea vulgaris</i>	Muscle 0.028 Liver 0.22	Muscle 0.05 Liver 0.40	Muscle 7.81 Liver 21.3	Usero <i>et al.</i> , (2002)
Southern Atlantic coast of Spain (Bacuta)	<i>Anguilla anguilla</i>	Muscle 0.032 Liver 0.48	Muscle 0.09 Liver 0.60	Muscle 11.4 Liver 37.0	Usero <i>et al.</i> , (2002)
Southern Atlantic coast of Spain (Bacuta)	<i>Liza aurata</i>	Muscle 0.030 Liver 0.51	Muscle 0.03 Liver 0.32	Muscle 6.07 Liver 49.6	Usero <i>et al.</i> , (2002)
Gölbaşı (Turkey)	<i>Clarias gariepinus</i>	Muscle < 0.001 Liver < 0.001 Gill < 0.001	Muscle 0.014 ± 0.005 Liver 0.052 ± 0.015 Gill 0.035 ± 0.014	Muscle 0.456 ± 0.098 Liver 4.392 ± 0.837 Gill 0.921 ± 0.154	Türkmen <i>et al.</i> , 2006
Gölbaşı dam (Turkey)	<i>Carasobarbus luteus</i>	Muscle 0.002 Liver 0.002 Gill 0.003	Muscle 0.008 ± 0.001 Liver 0.058 ± 0.014 Gill 0.017 ± 0.002	Muscle 0.286 ± 0.018 Liver 1.363 ± 0.086 Gill 0.685 ± 0.020	Türkmen <i>et al.</i> , 2006
Black sea (Turkey)	<i>Scorpaena porcus</i>	Muscle 0.80 ± 0.06	Muscle 0.66 ± 0.06	Muscle 95.3 ± 8.7	Uluozlu <i>et al.</i> , (2007)
Black sea (Turkey)	<i>Mullus barbatus</i>	Muscle 0.45 ± 0.04	Muscle 0.84 ± 0.07	Muscle 106 ± 9.1	Uluozlu <i>et al.</i> , (2007)

10.6 $\mu\text{g/l}$; LOD (Zn) = 18 $\mu\text{g/l}$. The average concentration of Ca and P were respectively 531.3 ± 63.76 mg/l and 3.39 mg/l.

4 Sediment

The concentration of Pb, Zn, P and Ca were respectively 38.01 ppm; 640.60 ppm; 452.60 ppm and 28.4 %. The Cd concentration in sediment was below the limit of detection (LOD = 1.0 ppm).

DISCUSSION

In this study, three types of spinal deformities consisting of a kyphosis, lordosis and scoliosis which frequently co-occur in varying degrees of severity are described, to our knowledge for the first time, in *A.*

lagunae from the Tunis North Lake (Tunisia) (Ayed *et al.*, 2005; 2006, 2008 and 2009). These deformities were similar to those described in *Z. ophiocephalus* from the Karin Sea Eastern Middle Adriatic (Dulcic 2004), in *Atherina boyeri* from the estuary of the Neretva (Tutman *et al.* 2000) and reared *Sparus aurata* (Afonso *et al.*, 2000). Spinal deformities in *A. lagunae* were found to be significantly associated with fish length. In both sexes, the highest occurrence of deformities was observed in the 20–25 mm class decreasing thereafter with fish length. The same result was obtained by Antunes and Lopes Da Cunha (2002), who showed, in *Gobius niger* from Sado estuary in Portugal, that vertebral deformities were more frequent in the 60–69 mm class decreasing thereafter with fish length. These results also agree with those reported by Tutman *et al.*, (2000), who

noticed a decrease in spinal deformities with age in *A. boyeri*. The absence of older deformed fish could be explained by higher mortality of deformed fish as compared to normal fish. In fact, different possible effects on the individual due to spinal deformities are described in the literature: impaired swimming performance (Weiss and Weiss, 1976), decreased ability to escape (Kroger and Guthrie, 1971) and hindrance to catching food (Kroger and Guthrie, 1971).

Many environmental factors can cause spinal deformities in wild fish populations, making detection of causal relationships difficult. The skeletal deformities can be environmentally induced by alteration of biological processes necessary for maintaining the biochemical integrity of bone, or neuromuscular effects, which lead to deformities without a chemical change in vertebral composition (Lall and Lewis-McCrea, 2007). Our assessment of factors that may be causing the high incidence of deformities in sand smelt populations was based on comparisons of calcium and phosphorus, and some contaminants (Cd, Pb and Zn), with published information on relationships between environmental variables and fish deformities. Several physiological, environmental, genetic, xenobiotic and nutritional factors have been linked to this problem during larval and juvenile development stages of cultured freshwater and marine fish. These factors include phosphorus and calcium deficiencies and toxicities (Kessabi *et al.*, 2009). Skeletal abnormalities can also be caused by genetic factors such as mutations, hybridization or inbreeding (Madsen and Dalsgaard, 1999). Generally deformities are a complex mixture of different bone disorders including vertebral and spinal malformations such as kyphosis, lordosis and scoliosis. The frequency and severity of skeletal abnormalities can be directly related to age, as noted in halibut (Lewis *et al.*, 2004), while an indirect relationship has been observed in sharpnose seabream (Favaloro and Mazzola, 2000). Calcium, zinc and phosphorus either promote bone formation or mineralization (Lall and Lewis-McCrea, 2007). Calcium and phosphorus are closely related to the development and maintenance of the skeletal system and the stability of the vertebrae are maintained by a solid phase of calcium phosphate. Fish and other aquatic organisms absorb Ca and P from water and their Ca requirement is met by their ability to absorb this element directly from water. Unlike terrestrial animals, bone is not the major site of Ca regulation in fish. Fish gills provide them with continuous access to an unlimited Ca reservoir and Ca regulation occurs at the gills, fins, and oral epithelia tissues. The absorbed calcium is deposited in bone, scale and skin (Lall and Lewis-McCrea, 2007). In this study, the concentrations of Ca in gills were significantly lower in deformed fish than in healthy ones (50.401 ± 6.738

mg/g dry weight in atherines deformed and 62.085 ± 1.939 mg/g dry weight in normal atherines). Calcium deficiency is not common in fish. Phosphorus deficiency signs include reduced growth, decreased feed efficiency, reduced bone mineralization and skeletal abnormalities. The concentrations of phosphorus in deformed atherines were lower in all samples analyzed. The phosphorus liver concentrations in healthy specimen were twice that those deformed. This differences was highly significant ($p < 0.01$).

The concentrations of metals in the liver represent the storage of metals from the water where the fish species live (Romeo *et al.*, 1999; Karadede *et al.*, 2004). Thus, the liver is more often recommended as environmental indicator organ of water pollution than any other fish organs. We have found that Zn concentrations in muscle, gonads, gill and in liver of deformed *A. lagunae* from the Tunis North Lake were significantly higher than normal atherina. Zn in muscle of *Atherina lagunae* exceeds the values found in *Sardina pilchardus* (34.58 $\mu\text{g/g}$) and *Atherina hepsetus* (24.34 $\mu\text{g/g}$) (Canli and Atli, 2003), *Sarda sarda* (48.7 $\mu\text{g/g}$) (Uluozlu *et al.*, 2007); *Liza ramada* (12.28 $\mu\text{g/g}$); *Shelon labrax* (7.24 $\mu\text{g/g}$) *Mugil cephalus* (40.2 $\mu\text{g/g}$); *Lithognathus mormyrus* (5.83 $\mu\text{g/g}$) (Uysal *et al.*, 2008). The liver concentrations were higher than those found in *Carasobarbus luteus* (1.36 $\mu\text{g/g}$) and *Clarias gariepinus* (4.39 $\mu\text{g/g}$) (Türkmen and Ciminli, 2007). High values of Zn were reported in *L. aurata* (200.90 $\mu\text{g/g}$), *C. labrax* (190.20 $\mu\text{g/g}$), *M. cephalus* (161.30 $\mu\text{g/g}$), *S. aurata* (265.20 $\mu\text{g/g}$) and *L. ramada* (339.79 $\mu\text{g/g}$) (Uysal *et al.*, 2008). However, other studies show concentrations of Zn in the gills very low in *Carasobarbus luteus* (0.685 $\mu\text{g/g}$) and *Clarias gariepinus* (0.924 $\mu\text{g/g}$) (Türkmen and Ciminli, 2007). Considering the heavy metal concentrations in the liver, the deformed fish exhibited higher accumulated liver concentrations of Zn indicative of a differential ability to handle the metal. In accordance with the present study, Bengtsson *et al.* (1985) have found high frequency of vertebral deformities in *Myoxocephalus quadricornis* exposed to heavy metal pollution in the Gulf of Bothnia (Baltic Sea). Bengtsson (1974) suggested that vertebral damage caused by Zn might be attributed to its effect on the muscle action potential and thus to an effect on the neuromuscular system. In fact, field surveys (Valentine, 1975; Bengtsson, 1979, 1988; Bengtsson *et al.*, 1985; Boglione *et al.*, 2006) provide evidence of environmental influence on induction of skeletal abnormalities, including spinal and vertebral deformities. A causal relationship between high environmental levels of chlorinated hydrocarbons or heavy metals and skeletal anomalies in fish has been suggested (Valentine, 1975; Mehrle *et al.*, 1982; Bengtsson *et al.*, 1985; Bengtsson, 1988). Induction

of spinal deformities in fish can be confounded by a number of biotic factors (e.g. hereditary defects, parasite infections) and abiotic factors (e.g. vitamin deficiencies, electrical shock. . .) which are not related to pollution. Cadmium (Cd) is considered to be one of most toxic heavy metals. It enters the environment from natural and, essentially, anthropogenic sources (Burger, 2008). Cd dissolved in water or deposit in sediment constitutes a contamination source for the various aquatic food chain links (Romeo and Gnassia-Barelli, 1995). On the basis of the obtained results and the above mentioned requirements we propose to use the incidence of spinal deformities in *A. lagunae* as a biomarker for future monitoring programs, to evaluate the evolution of heavy metal pollution in the Tunis North Lake. However, this study must be regarded as a preliminary approach to this problem, due to the low number of sites examined.

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