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Accumulation and Tissue Distribution of Domoic Acid in the Common Cuttlefish, Sépia Officinalis from the South Moroccan Coast.

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Abstract

Domoic acid (DA) is a phycotoxin produced by some diatoms, mainly from the Pseudo-nitzschia genus, and has been detected throughout the marine food web. In Morocco, many mollusc species are subject to regular monitoring of levels of contamination by toxins via Network Observation of the safety of the Moroccan coast (RSSL) implemented by National Fisheries Research Institute (INRH). Among these toxins, AD which has been frequently found in the bivalve molluscs, little known about DA accumulation in cephalopod. This study presents the first data showing concentrations of DA that exceed health limits detected in the common cuttlefish, Sepia officinalis from the south Atlantic coast of Morocco. Domoic acid was found throughout 2014 and 2015 in the digestive gland and flesh of cuttlefish reaching concentrations of 50 mg DA kg-1.

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The highest DA values were detected during autumn month. Evaluation of DA tissue distribution showed elevated DA concentrations in the digestive gland. The common cuttlefish, like other cephalopod species, plays a central position in the food web and might be a new DA vector to top predators like marine mammals. Human intoxications are not expected as long as DA was only detected in the flesh at levels (16 mg DA kg-1) not exceed regulatory value. However, in some countries, whole juvenile animals are consumed (without evisceration), and in this case they might represent a risk to human health as the AD accumulation is more significant in the digestive gland than in the flesh. This study reveals a new member of the marine food web able to accumulate DA in Morocco.

Keywords: Domoic acid; Sepia officinalis; Amnesic shellfish poisoning.

1. Introduction

Cephalopods constitute a class of marine molluscs that are found in a wide variety of habitats. They are active predators, feeding on a large range of live prey, and their high growth and metabolic rate [1-3] make them important in terms of impact on the food web [4]. The diet of sepia officinalis specie consists mainly of crustaceans, fish and occasionally molluscs including bivalves [5-7]. These groups of prey may consider as domoic acid vectors [8-10].

In the southern Moroccan Atlantic area, cephalopods are a very important fishery potential after fish. Among the cephalopod species interesting figure cuttlefish Sepia officinalis, this is the second target species by the national fleet after the octopus Octopus vulgaris.

Domoic acid was identified as the toxin responsible for an outbreak of human poisoning that occurred in Canada in 1987 following consumption of contaminated blue mussels (*Mytilus edulis*) [11]. The poisoning was characterized by a constellation of clinical symptoms and signs. Among the most prominent features described was memory impairment, which led to the name Amnesic Shellfish Poisoning (ASP). Certain marine organisms, such as the red alga Chondria armata and planktonic diatom of the genus Pseudo-nitzschia, produce Domoic acid. Since 1987, monitoring programs have been successful in preventing other human incidents of ASP. However, there are documented cases of domoic acid intoxication in wild animals and outbreaks of coastal water contamination in many regions worldwide. In Morocco, the presence of DA with abnormal levels has been recorded in the blue mussels (mytilus edulis) from North Atlantic coast [12]. Hence, domoic acid continues to pose a global risk to the health and safety of humans and wildlife. Several mechanisms have been implicated as mediators for the effects of domoic acid.

The mechanism of domoic acid toxicity is explained by its structural similarity with the excitatory neurotransmitter glutamic acid and its analogues (Figure 1), but with a much stronger receptor affinity. Domoic acid is three times more potent than its analogue kainic acid and up to 100 times more potent than glutamic acid itself [13]. After exposure, domoic acid binds predominately to N-methyl-D-aspartate (NMDA) receptors in the central nervous system [14], causing depolarization of the neurones. Subsequently, the intercellular calcium concentration increases, resulting in sustained activation of calcium sensitive enzymes, eventually leading to

depletion of energy, neuronal swelling and cell death. Since the Canadian incident 11 species of Pseudo-nitzschia and 1 species of Nitzschia have been shown to produce this neurotoxin [15-17], which may accumulate in filter-feeding bivalves. Although bivalves were the vectors in the first ASP episode and a regulatory value for them (20 mg DA/kg whole soft tissue) was established, subsequent DA poisoning events have revealed that many other marine organisms could also be vectors.

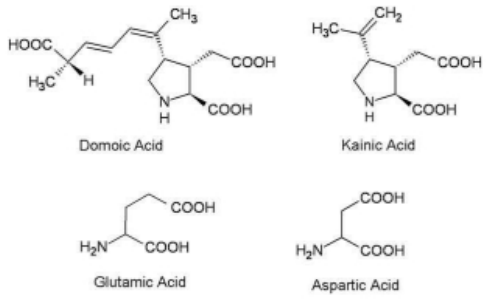

Figure 1: Chemical structure of domoic acid and related amino acids

Recent studies in California indicate that planktivorous fish are even more effective vectors than bivalves [18,19]. Thus, domoic acid is currently considered a transferred algal toxin that, through feeding interactions in marine food webs, can cause vectorial intoxication of consumers at higher trophic levels. Accordingly, DA has been detected from the small and simple herbivorous organisms such as copepods and krill to the top predators as marine mammals and sea birds [20-24].Cephalopods which are important members of the food chain, and active predators of known toxin vectors such as bivalves, crabs and planktivorous fish, have just recently been implicated in DA transfer and accumulation, when high concentrations of DA were detected in the digestive gland of the common octopus (Octopus vulgaris) [10] et common cuttlefish, Sepia officinalis [25].

The common cuttlefish, Sepia officinalis, is a nektobenthic species. It is a demersal , neritic who likes mixed funds , sandy , muddy or herbarium the coast to about 150 m depth but is more abundant above 100 m [26, 27].

The main aims of this work were to study the presence of DA in the common cuttlefish, Sepia officinalis from the Moroccan coast, describe its accumulation during 2-year period, evaluate and compare the tissue distribution of this toxin in digestive gland and flesh (mantle) and the relationship between toxin accumulation and cuttlefish weight, or size.

2. Materials and methods

2.1. Collection and preparation of cuttlefish samples

Four samples of Sepia officinalis comprising a total of 164 individuals, were captured by small scale fishing in Lassarga located at the tip of the peninsula of Dakhla, regent in southern of Morocco , and by trawling scientific campaigns for the prospecting of cephalopods using the R/V " Sharif Al Idrissi " in the southern Moroccan Atlantic area between Cap Boujdor (CBj) (26°N) and Cap Blanc (CB) (20°50'N) (Figure 2) during the period between July 2014 et May 2015 (Tableau 1). All cuttlefish samples were kept at −20◦C until use. The dissection, was carried out under partially defrost conditions and without rupture of the outer membrane of

the organs in order to minimize DA leakage and contamination across tissues.

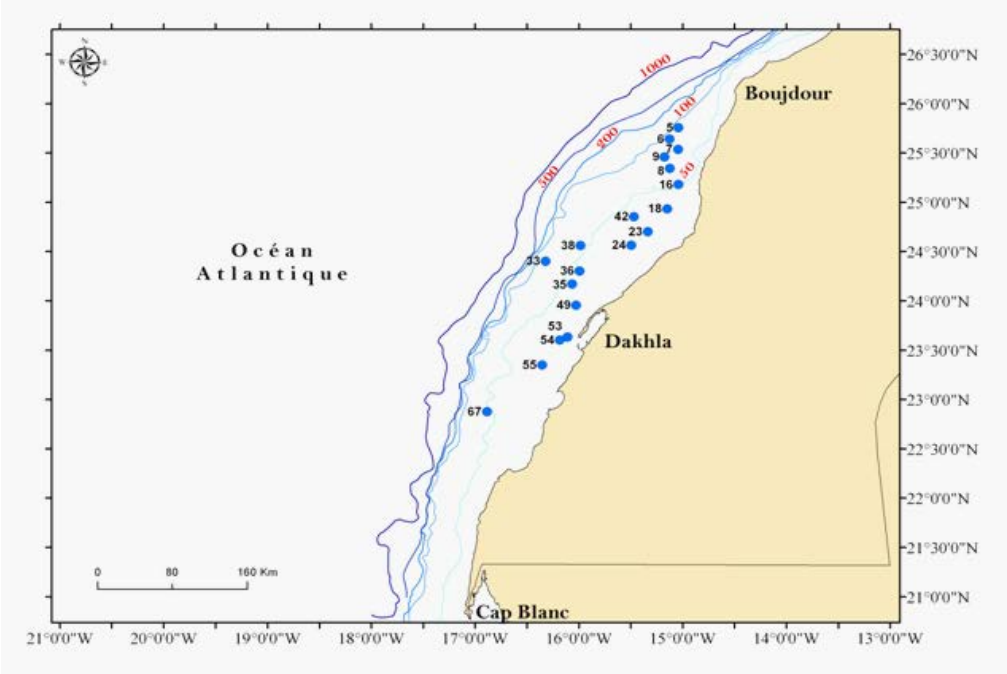

Figure 2: Distribution of sampling stations of Sepia during October 2014 by for the prospecting of cephalopods in southern Moroccan coast.

Table 1: Sepia officinalis: description of samples collected in the southern Moroccan coast, (n = number of individuals)

Sample	Date (mois/année)	Fishing nature	Depth (m)	Cuttlefish weight (g, mean ± SD)	Cuttlefish size (cm, mean ± SD)	n
C1	07/2014	Scale fishing	10-30	575,93± 421,60	16.43± 4.9	34
C2	09/2014	Scale fishing	10-30	506,5± 189	16.9± 2.43	43
C3	10/2014	Prospecting campaign	18-100	615,8± 462,9	16.38± 4.08	45
C4	05/2015	Prospecting campaign	18-100	859,23± 572	18.95± 4.51	42

The biometric measurements were taken: The dorsal mantle length (Ldm) in centimeters and the whole weight (P) in the gram.

To study the tissue distribution of DA, specimens of the all samples collected in 2014-2015 were dissected into the digestive gland and the flesh (mantle).

These tissues were individually homogenized and a 5 g portion of each was weighed separately.

2.2. Toxin extraction and HPLC analysis

Extractions were carried out according to the method [28]. The extraction was performed with aqueous 50% methanol at 10000 rpm with a homogenizer probe for 3 min, centrifuged at 4000 g for 10 min and filtered a proportion of the supernatant through a dry methanol-compatible 0.2 µm filter.

The equivalent of 20 µl was injected on the column without any further clean-up. Liquid chromatography was performed on a Shimadzu Model LC 20AD equipped with inline degasser (DGU-20A), pump of isocratic elution (LC-20AD), auto sampler (SIL-20AC), oven (CTO-20A) and the HP Lab solutions software performed diode-array detector (SPD-M20A); data collection and results treatment.

The column used was C-18 a reversed phase (250 mm×4.6 mm, 5µm), with a guard-column at 1 ml min−1.

Detection wavelength was set at 242 nm with a 10 nm bandwidth. Calibration was carried out using DACS-1D certified DA standard, purchased from the National Research Council of Canada (NRC). Under these conditions, the detection limit was 0.04 µgml−1, corresponding to 0.2 mg kg−1 in tissue Lichrospher 100 RP-18 (4mm×4 mm, 5µm), both heated to 40◦C±2°C. The flow rate was set at 1ml/min. For statistical analyses, the Mann–Whitney test was applied for comparison between DA isomer levels in the digestive gland and flesh, and the Pearson correlation test was used. The XLSTAT was used.

3. Results

From the extract of the digestive gland of Sepia officinalis, the compound DA observed eluting at 6.5 minutes in the HPLC chromatogram (figure 2). Its retention time matched well with the retention time of the calibration standard (Figure 3). Other peaks eluting close to DA were identified as their isomers, namely isodomoic D (iso-D) and epidomoic (epi-DA). In the UV spectrum, the iso-D and epi-DA peaks had maxima at 244 and 242 nm.

The peak identified as triptophane (TRP), which could cause interferences and require clean-up procedures, was eluted about 2 min earlier than the DA peak.

The UV/diode-array spectrum of the DA peak (maximum at 242 nm) from the cuttlefish tissues extract matched (>99%) with the spectrum acquired for the DA standard (Figure 4).

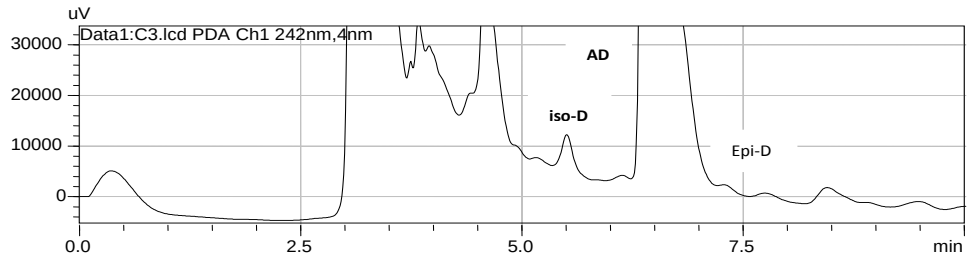

Figure 3: Chromatogram (wavelength 242 nm) obtained fromLC-UV analysis of a digestive gland extract of sepia officinalis from sample C3 (DA = 50mg Kg−1).

DA was detected in sepia officinalis, with the highest DA value registered in digestive gland for sample C3 collected during 2014 occurred in autumn when a maximum concentration of 50 mg DA/kg surpassing 20 mg DA/kg was recorded (Tableau 2). When analyzed individually, only in 57% of the sepia officinalis specimens was detected DA in digestive gland and only in 56% specimens was detected in flesh (mantle). The average value of DA concentration in the mantle of specimens from all samples was 3 mg/kg and 13.5 mg/kg. This maximum concentration of 13.5 mg/kg was found in the sample C3 (Figure 6) and (tableau 2).

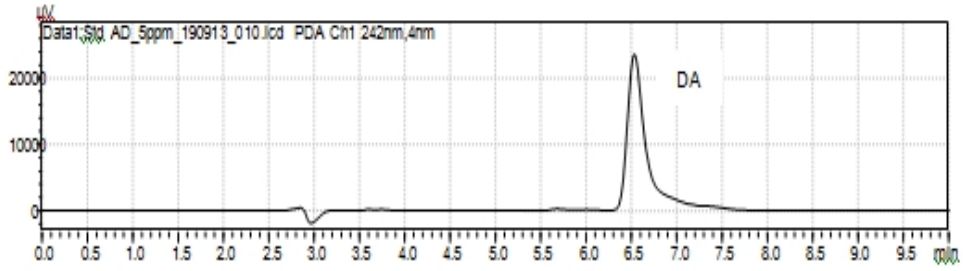

Figure 4: Chromatogram (wavelength 242 nm) obtained from LC-UV of certified standard (DA domoic acid).

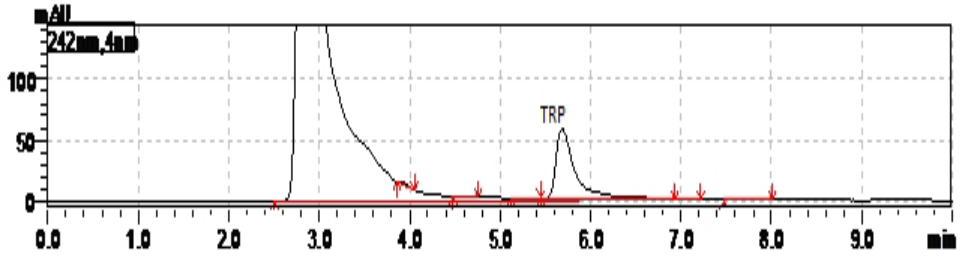

Figure 5: Chromatogram (wavelength 242 nm) obtained from LC-UV analyzes of certified Tryptophan (TRP).

Table 2: Domoic acid concentration (mg kg^{-1}) detected in the digestive gland and flesh of sepia officinalis from the southern Moroccan coast (ND = not detected; DG= digestive gland; FL= flesh. B j= Boujdour; CB = Cap Blanc).

Sampling location	Samples	Date (mois/année)	Depth (m)	Sepia officinalis weight (g, mean+SD)	No of individuals	Domoic acid (mg kg−1)			
						Mean		[min-max]	
						DG	FL	DG	FL
Lassarga	C1	07/2014	0-30	575.95± 421.60	34	3.8	0.6	[ND-10.6]	[ND -6.7]
Lassarga	C2	09/2014	0-30	506.5±189	43	2.13	0.76	[ND -11.3]	[ND -14]
Bj-CB	C3	10/2014	0-100	615.85±462.9	45	5.12	0.70	[ND −50]	[3 -13.5]
Bj-CB	C4	05/2015	0-100	506.5±189	42	2.4	1.18	[ND -8.4]	[ND-6.4]

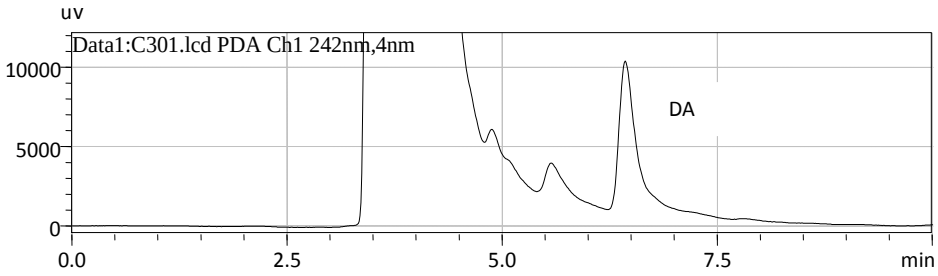

Figure 6: Chromatogram (wavelength 242 nm) obtained from LC-UV analysis of a mantle extract of sepia officinalis from sample C3 (DA = 13.5 mg Kg−1).

The tissue distribution of the toxin was evaluated in specimens from all samples and a significant difference DA accumulation was observed in digestive gland and Flesh (mantel) (p-value < 0.05) (figure 7). In other words, the DA contamination is greater in the digestive gland than in flesh (sided p-value = 0.001).

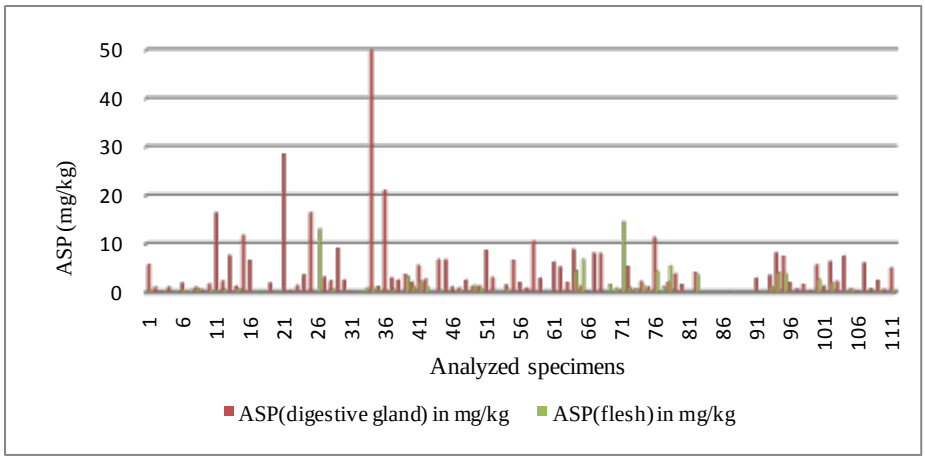

Figure 7: Evolution of the DA in the digestive gland and in the flesh of sepia officinalis specimens from the Moroccan coast during 2014-2015.

The study of relationship between domoic acid concentration in digestive gland and cuttlefish size or weight has disclosed a negative correlation (p-value > 0.05) (Figure 8).

4. Discussion

During the examination of the common cuttlefish (Sepia officinalis), domoic acid (DA) was frequently detected although it is a hydrophilic nature toxin that makes it more likely to be depurated than accumulated as is generally the case for the majority of bivalve species [29-32]. However, DA was constantly found in the digestive gland of S. officinalis during 2014 and 2015 reaching concentrations at 50 mg DA/kg, which shows this species to be a potential DA vector. Similar results of DA retention were reported for another cephalopod species, the common octopus (Octopus vulgaris) [10] and the common cuttlefish (Sepia officinalis) [25].

DA retention has been reported for other molluscs different feeding strategies such as the king scallop (Pecten maximus) and bivalve mollusk where high DA concentrations are found in the hepatopancreas [33, 34].

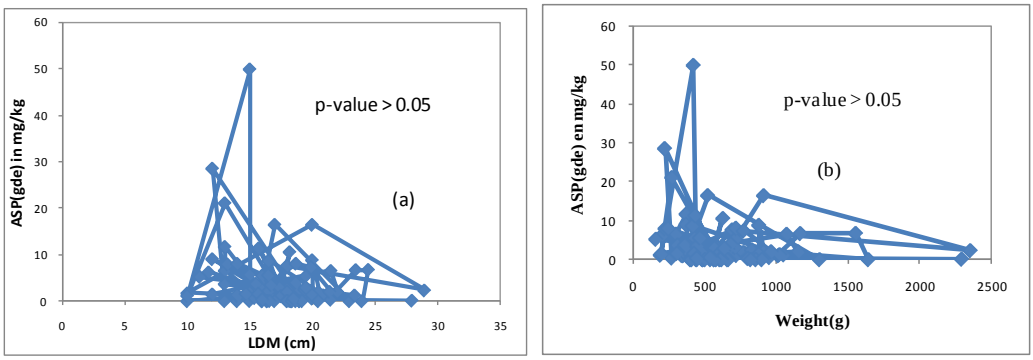

Figure 8: Dispersion ASP (digestive gland) data and LDM data of cuttlefish specimens (a) and dispersion ASP (digestive gland) weight data of cuttlefish specimens (b).

In cephalopods, the hepatopancreas or digestive gland, similarly to other molluscs, is a site of digestion and digestive absorption, and acts as a reservoir of prey material [35,36] that accumulates substantial reserves throughout sexual maturation (proteins 50-60% dry weight, lipids 10-25% dry weight; [37]).

Most of the specimens mature or immature, male or female present the considerable high variability in DA levels detected did not permit establishment of the correlation between toxin accumulated and cuttlefish size or weight. This is, has been confirmed by [25].

Sépia officinalis has a wide geographic and bathymetric distribution over the shelf regions of the whole coast, but more abundant in the Atlantic Ocean to the southern coast of Morocco [38] and found in shallower waters (in this study from the 18 to 100).

In the Atlantic Moroccan coast, upwelling events are responsible for the occurrence of algal blooms, including DA-producing Pseudo-nitzschia species that bloom in high densities in the surface layers of the ocean. Domoic acid associated with these blooms accumulates in planktivorous organisms that filter-feed on the surface waters. Cuttlefish are carnivorous, not filter feeders and they cannot obtain DA directly. Therefore, it is evident that the highest concentration exceeding 20 mg DA/kg, measured in October 2014, in cuttlefish digestive glands were an indirect result of pseudo-nitzschia blooms that occurred about some weeks earlier because in most of cases, the high densities of Pseudo-nitzschia species in the Moroccan coast occur in spring and summer months. The same bloom Pseudo-nitzschia periods observed in the Portuguese coast [39, 40, 10]. The toxin uptake by cuttlefish should be associated with their feeding habits and, like many cephalopods; cuttlefish are active predators with an intense metabolism, high food and high digestion efficiencies [41].

On the Portuguese coast, the cuttlefish diet is comprised mainly of fish and crustacean [42- 45]. In the Moroccan

coast, the same cuttlefish diet nature was found [46]. These preys can act as DA vectors [8-10], which results in sporadic uptake of toxin and bioaccumulation in the cuttlefish digestive gland. Alternatively, towards the end of Pseudo-nitzschia blooms, diatoms cells can sink to the seafloor where they can remain alive for a short period [47, 48]. Since benthic cephalopods live in direct contact with the sub strum, and many nectobenthic species such as cuttlefish spend long periods buried into sediment, benthic sources for DA uptake should be also considered.

The anatomical distribution of the amnesic toxin in both digestive gland and mantle showed elevated DA concentrations in the digestive gland. DA isomers were detected in this tissue (epi-DA and iso-D, respectively). Iron levels in Sepia officinalis tissues are high in digestive gland [49, 50] and could be co-responsible for the degradation products. The digestive gland has important functions in system detoxification because he accumulates high levels of toxins, and transforms them to less toxic derivatives. Thus, intoxication of other organs like mantle of the cephalopod is minimized [51, 52] .A low concentration of DA detected occasionally in mantle might be a result of contamination from the toxic tissue (digestive gland). Nevertheless, the cuttlefish mantle was almost salubrious of toxin revealing that DA leakage and contamination across tissues if happened was not very considerable. Contrary to what was observed in a species of Australian octopus that accumulates saxitoxin (paralytic shellfish toxin) in its legs [53], cuttlefish do not present any new health hazard to humans because DA concentration in mantle, the edible part, did not exceeding the limits. However, individuals smaller are consumed without evisceration in Portugal and other countries of which Morocco could be one who practices this form of consumption. In this case, cuttlefish may be potential vectors of DA to humans. Nevertheless, the Sepia officinalis diet is different for the smallest and largest animals [45], thus exposures to DA sources should also be different.

5. Conclusion

In Morocco, this is the first report of DA detected in Sepia officinalis, a cephalopod species that plays an important role in the marine food web and ocean ecology. Further studies of juvenile cuttlefish individuals are needed. No relationship between toxin accumulation and cuttlefish size or weight was found. The study of DA occurrence in the other species, such as S. elegans and S. orbignyana, could also be of great importance for a better understanding of the presence of the amnesic shellfish poisoning toxin in these mollusc categories other than bivalve molluscs.

In all of the sepia individuals subjected to the analysis of the ASP, the concentrations of AD having exceeded the threshold sanitary which is 20 mg / kg have been found only in the digestive gland , non edible portion by the consumer. The noble part flesh (mantel) remains safe, and poses no health risk.

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