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Estimation of Mercury Levels in Non-Edible Tissues (viscera and Brain) of the Common Octopus from the Western Tripoli Coast

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Abstract:
This study aimed to evaluate mercury (Hg) accumulation in tissues of the common octopus (*Octopus vulgaris*) collected from two distinct sites with contrasting levels of environmental contamination: Zawia Refinery and Marsa Zawagha-Sabratha. A total of *5* specimens were collected from Zawia Refinery and *3* specimens from Marsa Zawagha-Sabratha. Tissue samples, including viscera and brain, were analyzed to determine Hg concentrations. Octopus viscera from the Zawia Refinery exhibited Hg levels ranging from 0.052 to 0.165 mg/kg, with the highest concentration observed in the largest individual weighing 1.5 kg. In contrast, viscera from Marsa Zawagha-Sabratha showed substantially lower Hg levels (0.0285–0.0329 mg/kg). Brain tissue analysis revealed even higher Hg accumulation than viscera in the same locality, with concentrations ranging from 0.11 to 0.31 mg/kg in Zawia, whereas brain Hg levels in Marsa Zawagha-Sabratha were minimal (0.01–0.0121 mg/kg). The study also demonstrated a size-dependent trend, with larger octopus's individuals accumulating higher Hg concentrations in viscera, reflecting prolonged exposure and increased dietary intake over time. A direct comparison between organs indicated selective tissue accumulation, as Hg concentrations in the brain were nearly double those in the viscera, suggesting preferential retention in protein- and lipid-rich neural tissues, which may facilitate biomagnification. These findings highlight the significant influence of local environmental conditions, particularly proximity to industrial discharges, on Hg bioaccumulation patterns in octopus. The results emphasize the need for tissue-specific monitoring of heavy metals in marine organisms and stricter control of industrial pollution to safeguard seafood safety and marine ecosystem health.

Keywords: Octopus Vulgaris, Mercury, Viscera, Brain.

تقدير مستويات الزئبق في الأنسجة غير الصالحة للأكل (الأحشاء والدماغ) للأخطبوط الشائع من الساحل الغربي لطرابلس

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الملخص
هدفت هذه الدراسة إلى تقييم تراكم الزئبق (Hg) في أنسجة الأخطبوط الشائع (*Octopus vulgaris*) المأخوذ من موقعين متباينين من حيث التلوث البيئي: مصفاة الزاوية ومرسى زواغة - صبراتة تم جمع 5 عينة من الأخطبوط من منطقة مصفاة الزاوية و3 عينة من مرسى زواغة- صبراتة . شملت العينات المدروسة الأحشاء والدماغ لتحديد تراكيز الزئبق. أظهرت أحشاء الأخطبوط من منطقة مصفاة الزاوية تراكيز Hg تتراوح بين 0.052 و0.165 ملغم/كغم، مع تسجيل أعلى قيمة في أكبر فرد يزن 1.5 كغم، بينما أظهرت أحشاء العينات من مرسى زواغة-- صبراتة مستويات أقل بكثير (0.0285–0.0329 ملغم/كغم). وكشفت تحليلات الدماغ عن تراكم أعلى للزئبق مقارنة بالأحشاء في نفس الموقع، حيث تراوحت تراكيز Hg في الدماغ من 0.11 إلى 0.31 ملغم/كغم في الزاوية، بينما كانت مستويات Hg في الدماغ من مرسى زواغة - صبراتة منخفضة جدًا (0.01–0.0121 ملغم/كغم). أظهرت الدراسة أيضًا علاقة بين الحجم وتراكم الزئبق، حيث يميل

الأفراد الأكبر حجمًا إلى تراكم مستويات أعلى في الأحشاء، مما يعكس التعرض الطويل وزيادة المدخول الغذائي للمعادن الثقيلة مع الوقت. وأوضحت المقارنة المباشرة بين الأعضاء تراكمًا انتقائيًا في الأنسجة، إذ كانت تراكيز الزئبق في الدماغ تقريبًا ضعف تلك المسجلة في الأحشاء، ما يشير إلى احتفاظ تفضيلي في الأنسجة الغنية بالبروتين والدهون، الأمر الذي قد يسهل تكبير التضخّم الحيوي داخل الكائن الحي. تسلط هذه النتائج الضوء على الدور الكبير للظروف البيئية المحلية، وخصوصًا القرب من المخلفات الصناعية، في تحديد أنماط تراكم الزئبق في الأخطبوط.. تؤكد النتائج أهمية مراقبة تراكم المعادن الثقيلة في الأنسجة المختلفة للأحياء البحرية وضرورة التحكم الصارم في التلوث الصناعي للحفاظ على سلامة الغذاء وصحة النظم البيئية البحرية.

الكلمات المفتاحية: الأخطبوط الشائع، الزئبق، الأحشاء، الدماغ.

Introduction

Marine organisms, particularly cephalopods such as octopus and squid, constitute an essential component of global seafood resources because of their high nutritional value and increasing market demand. Nevertheless, the marine environment is continuously subjected to industrial and anthropogenic pollution, which in turn leads to the bioaccumulation of toxic heavy metals—including mercury (Hg), cadmium (Cd), and lead (Pb)—in marine food webs. Consequently, these contaminants may pose substantial health risks to humans who consume contaminated seafood (Nessim & Riad, 2003). In line with these concerns, numerous studies have documented elevated levels of heavy metals in edible marine species across various regions. Notably, Storelli and Marcotrigiano (2004) reported significantly high concentrations of Hg and Cd in the flesh and hepatopancreas of albacore tuna (Thunnus alalunga) and horned octopus (Eledone moschata), with several samples surpassing the maximum allowable limits established by the European Commission. Furthermore, research focusing specifically on the common octopus *Octopus vulgaris* has demonstrated tissue-specific bioaccumulation trends, whereby metal concentrations are markedly higher in the digestive gland compared to the muscular mantle. Such variation has been attributed to differences in environmental contamination and mercury bioavailability between coastal sites (Raimundo et al., 2010; Seixas et al., 2005). Additionally, Storelli et al. (2006) found that a considerable proportion of tested cephalopod specimens—particularly those from polluted regions—exhibited Cd and Hg levels exceeding regulatory safety limits, especially in non-edible organs such as the hepatopancreas. These findings highlight a potential risk to consumers who frequently include cephalopods in their diet. Similarly, Ariano et al. (2019) demonstrated that cephalopods captured from highly industrialized coastal zones may contribute significantly to dietary exposure to toxic metals, although differences between organs and sampling locations remain statistically relevant. On the other hand, studies conducted in less impacted marine environments, such as Peninsular Malaysia, revealed that Hg levels in cephalopods are generally low and comply with international food safety standards, with no significant correlation observed between metal burden and organism size (Ahmad et al., 2015). Cephalopods, particularly the common octopus (*Octopus vulgaris*), represent an increasingly important segment of the global seafood market due to their high nutritional value and significant economic relevance (Ariano et al., 2019). Nevertheless, the ecological characteristics of these organisms—including their carnivorous feeding habits, rapid growth, and benthic lifestyle—render them susceptible to the bioaccumulation of environmental contaminants, especially toxic heavy metals such as cadmium (Cd), lead (Pb), and mercury (Hg). These contaminants can subsequently be transferred to humans through dietary exposure, posing potential health risks (Mok et al., 2014). Heavy metals originate from both natural processes and anthropogenic activities, including industrial discharge, maritime operations, and coastal urbanization. Consequently, these pollutants persist in marine ecosystems and biomagnify along food webs, causing higher trophic-level organisms to retain increasing concentrations throughout their lifespan (Minet et al., 2021). Moreover, cephalopods play a critical role in this bioaccumulation pathway, serving as major vectors for mercury transfer to marine predators. Previous studies have consistently reported tissue-specific patterns of heavy metal accumulation. For instance, internal organs—particularly the digestive gland or hepatopancreas—often exhibit significantly higher levels of Cd and Pb compared to muscular tissues, reflecting their metabolic functions in detoxification and nutrient processing (Storelli & Marcotrigiano, 1999; Mok et al., 2014). Similarly, Ariano et al. (2019) demonstrated spatial variability in Pb concentrations in the muscle of *O. vulgaris*, with specimens from Castellammare di Stabia showing higher levels than those from Naples, while Cd and Hg exhibited no significant geographic differences. Additionally, emerging evidence highlights the neurological implications of mercury exposure in cephalopods. Minet et al. (2021) documented elevated Hg concentrations in the brain tissues of certain benthic species, potentially affecting neural plasticity, behavioral functions, and ecological fitness. Such findings broaden concerns beyond food safety to include ecosystem health and species resilience. Although international regulatory agencies have established maximum permissible limits for heavy metals in seafood, research indicates that consumption of certain tissues—particularly internal organs—may exceed health-based guidance values, thereby elevating human exposure risk (Mok et al., 2014). Therefore, ongoing monitoring and risk assessment remain essential to ensure consumer protection, especially in coastal communities with high cephalopod consumption. This indicates that regional environmental conditions play a crucial role in influencing metal accumulation rates. Taken together, the scientific

evidence underscores the importance of continuous monitoring and assessment of heavy metal levels in cephalopods destined for human consumption. Therefore, investigating the bioaccumulation patterns of toxic metals in *Octopus vulgaris*—including inter-tissue variability and compliance with food safety regulations—remains essential for protecting consumer health and evaluating the ecological impact of marine pollution. Accordingly, the present study aims to quantify the concentrations of selected heavy metals in different tissues of common octopus's specimens and assess their compliance with international safety limits, thereby contributing to enhanced environmental management strategies.

Materials and Methods:

Materials:

Table (1): Chemical Reagents (Standard Solutions and Analytical Chemicals)

(Chemical Reagents (Standard Solutions and Analytical Chemicals)			
1	Standard solutions of mercury, arsenic, lead, and cadmium (E-Merck, Darmstadt, Germany)	2	Perchloric acid ($HClO_4$) at 70% concentration
3	Nitric acid (HNO_3) at 65% concentration	4	Hydrogen peroxide (H_2O_2) at 30% concentration
5	Hydrochloric acid (HCl) at 37% concentration	6	Deionized water
7	Distilled water	8	Acidified ultrapure water (5% v/v HNO_3)
9	Working standard solutions for calibration: - 0.005 ppm - 0.01 pm - 0.1 ppm - 0.2 ppm	10	Therand's solution, consisting of: - 100 ml of 37% HCl - 40 ml of 30% H_2O_2 - 125 ml of deionized water
11	Stock standard solutions of heavy metals (Hg, As, Pb, Cd) at 1000 mg/L	12	Blank solutions (prepared with HNO_3, HCl, $HClO_4$, H_2O_2, and deionized water)
13	Diluted acid solution, composed of: - 450 ml distilled water + 50 ml of 37% HCl		
Glassware and Laboratory Equipment			
	Glassware		Experimental containers
	Cleaning equipment (e.g., soaking tubs with soap and water)		Sanitized environment for drying the equipment
	Sterile screw-capped tubes		Sterile glass beakers
	Water bath (set to 53°C for overnight digestion)		Whatman filter paper No. 42
	Pipettes and measuring cylinders		Labeling materials (e.g., for sample numbers)
	Protective aseptic tools (for sample cutting and transfer)		
Analytical Instrumentation			
	Atomic Absorption Spectrophotometer (AAS) – used for measuring heavy metal concentrations		Calibration curve setup – based on prepared standard solutions

Methods:

Sample collection

A total of 8 octopus's samples were collected from two regions in western Libya. five samples of common octopus were collected from the beaches near the Zawiya Refinery, with five different weights for each group. And three samples were collected from the Sabratha region (Marsa Zawagha-Sabratha/Sabratha), located approximately 25 kilometers away from the first site, with three different weights for each group. The sampling took place during the period from March 1, 2024, to September 25, 2024. Each sample was individually packed in an impermeable plastic bag, labeled, and promptly transported to the Central Laboratory of Delta Technical Services Company (DSL) in Tripoli, Libya, where the samples were prepared and digested for heavy metals analysis.

Reagents and washing procedures

Standard solutions of mercury (E-Merck; Darmstadt, Germany) as well as 65% nitric acid, 70% perchloric acid, 37% hydrochloric acid, and 30% hydrogen peroxide were among the ultra-pure reagents used. Before being used, all glassware and lab equipment were submerged in soap and water for about two hours. After that, they were rinsed several times with tap water and again with distilled water to remove any last bits of dirt. The products

were then washed once using Therand's solution (100 ml concentrated HCL 37% + 40 ml H_2O_2 30% + 125 ml deionized water). Following that, they received a single wash with diluted acid (450 milliliters of distilled water plus 50 milliliters of 37% HCL). All experimental containers were finally rinsed with deionized water and then dried in a sanitized environment.

Samples preparation and digestion

The octopus's samples were digested using the wet digesting method described by Abd-Elghany et al. (2024). To put it briefly, two grams of each viscera and Brain octopus sample were aseptically cut, finely chopped, and placed in a sterile screw-capped tube along with four milliliters of 65% HNO3 and two milliliters of 70% HCLO4. After two minutes of vigorous shaking, the tube was left to sit at room temperature overnight. To guarantee thorough digestion, the tubes were heated to 53°C in a water bath for the duration of the night. The tubes were allowed to cool at room temperature before the digest was diluted with deionized water and filtered through Watt Man filter paper No. 42 into a sterile glass beaker. Finally, the filter was diluted with 50 milliliters of deionized water. The diluted filtrate was stored at room temperature in a sterile screw-capped tube labeled with the sample number prior to the measurement of the heavy metal concentrations.

To ensure the accuracy of the heavy metals analysis by atomic absorption spectrophotometry (AAS), the blank solutions (HNO_3, HCL, HCLO4, $H2O_2$, and deionized water) were also prepared using the same wet digestion method without the addition of sample. It is necessary to subtract any residual heavy metals from the results. By diluting a stock solution of 1000 mg/L of the metal under analysis (Hg) with acidified ultrapure water (5% v/v HNO_3), working standard solutions of 0.1ppm for the metal were created in order to construct the calibration curves.

Heavy metals analysis

Mercury (Hg) concentrations (mg/kg wet weight) were measured using the Buck Scientific USA 210 VGP model Atomic Absorption Spectrophotometer (AAS) at the Central Laboratory of Delta Technical Services Company (DSL) Tripoli, Libya, in accordance with AOAC (1990). The Hg was estimated using a flameless AAS equipped with Hg hydride system and a cold vapor method. The instrument was run at wavelengths of 253.7, nm and detection limits ranging from 0.005 to 0.2 mg/kg. Heavy metals concentrations in tested samples were estimated according to the following Equation:

Element (mg/kg wet weight) = R × D/W

Where (D) is the tested sample's dilution, (W) is the tested sample's wet weight, and (R) is the element concentration (mg/L) as read by the AAS digital scale. Additionally, the amount of metals in the blank solutions was measured and subtracted from each sample under examination.

Validation Method

Working standard solutions of 0.01, 0.05, 0.1, 0.5, 2, and 5 mg/L were made for mercury in order to construct calibration curves. The validation for heavy metals analysis was previously designated in details in a study performed by Abd-Elghany et al. (2024) through determining limits of quantification (LOQ) at 0.685 mg/kg, limits of detection (LOD) at 0.201 mg/kg, precision % (CV%) that equal 1.9 and % of spiking recovery at 96.4%. All analytical procedures employed in the current experiment were validated using a certified reference material (CRM), such as dogfish liver (DOLT-4, imported from Canada), yielding recovery values ranging from 97.7 to 104.7%.

Statistical analysis

This study investigates mercury (Hg) concentrations in Non-Edible Tissues of samples collected from two locations: Zawia Refinery and Marsa Zawagha-Sabratha. The results, expressed in mg/kg, are presented as mean ± standard deviation, along with mean ranks from a Mann-Whitney test to assess the statistical significance of differences between the two locations.

Results

Table (2): show concentration of Mercury (Hg) in octopus's viscera collected from the first area of the study (Zawia Refinery)

No	Sample ID	Organ	Weight of octopus	Mercury (Hg) (mg/kg)
1	S. B1	viscera	1.500kg	**0.165**
2	S. B2	viscera	1.200g	**0.14**
3	S. B3	viscera	710g	**0.077**
4	S. B4	viscera	500g	**0.06**
5	S. B5	viscera	260g	**0.052**

Table (3): show concentration of Mercury (Hg) in octopus's viscera collected from the second area of the study (Marsa Zawagha-Sabratha)

No	Sample ID	Organ	Weight	Mercury (Hg) (mg/kg)
1	S. B1	viscera	1.150kg	**0.0329**
2	S. B2	viscera	710gm	**0.0314**
3	S. B3	viscera	300gh	**0.0285**

Table (4): show concentration of Mercury (Hg) in octopus's brain collected from the first area of the study (Zawia Refinery)

No	Sample ID	Organ	Weight of octopus	Mercury (Hg) (mg/kg)
1	S. B1	Brain	1.500g	**0.31**
2	S. B2	Brain	1.100kg	**0.28**
3	S. B3	Brain	850g	**0.189**
4	S. B4	Brain	500g	**0.14**
5	S. B5	Brain	410g	**0.11**

Table (5): show concentration of Mercury (Hg) in octopus's brain collected from the second area of the study (Marsa Zawagha-Sabratha)

No	Sample ID	Organ	Weight	Mercury (**Hg**) **(mg/kg)**
1	S. B1	brain	1.100km	**0.0121**
2	S. B2	brain	455gm	**0.0109**
3	S. B3	brain	250gm	**0.01**

Discussion:

The data obtained in this study reveal pronounced differences in mercury (Hg) accumulation in *Octopus vulgaris* tissues between the two sampling areas (Zawia Refinery and Marsa Zawagha-Sabratha) as well as between organs (viscera vs. brain). In particular, octopus's viscera from the Zawia Refinery area (Table 2) exhibited Hg concentrations ranging from 0.052 to 0.165 mg/kg - the highest value corresponding to the largest specimen (1.5 kg). By contrast, viscera from Marsa Zawagha-Sabratha (Table 3) showed considerably lower levels (0.0285 to 0.0329 mg/kg). This disparity strongly suggests that local environmental conditions - likely linked to proximity to industrial discharge - significantly influence Hg bioaccumulation, a conclusion that echoes observations from earlier studies in anthropogenically impacted areas (Minet et al., 2021; Mok et al., 2014) as well as more recent investigations of cephalopods in polluted coastal zones (Viana et al., 2021; Cilli & Türk, 2024). A quantitative comparison highlights differences between our results and those reported in the literature. For instance, Viana et al. (2021) found that Hg concentrations in tissues of *Eledone cirrhosa* and other cephalopods reached up to approximately 1.89 $\mu g \cdot g^{-1}$ dry weight (≈1.89 mg/kg) in the optic lobes of the brain, which is substantially higher than the maximum brain Hg levels observed in our study (~0.31 mg/kg) despite species and environmental differences. In contrast, Cilli & Türk (2024) reported that no detectable accumulation of Hg occurred in the edible tissues of *Octopus* from Iskenderun Bay, indicating that Hg burdens in that region were low or below analytical detection limits, which aligns more closely with the comparatively low viscera Hg values at Marsa Zawagha-Sabratha-Sabratha. Additionally, these comparative data suggest that Hg levels in *O. vulgaris* from the Zawia Refinery are elevated relative to some less contaminated regions, but remain lower than the highest concentrations documented in cephalopod nervous tissues from heavily impacted environments. Moreover, within each area, a tendency for higher Hg concentrations in viscera of larger specimens was observed, aligning with known bioaccumulation dynamics in which older and larger individuals accumulate more heavy metals over time due to prolonged exposure and higher metabolic intake (Storelli & Marcotrigiano, 1999). Analysis of brain tissue yielded even more striking results: in samples from Zawia (Table 4) brain Hg ranged between 0.11 and 0.31 mg/kg, markedly exceeding concentrations recorded in Marsa Zawagha-Sabratha-Sabratha (0.01–0.0121 mg/kg) (Table 5). These findings suggest that mercury not only accumulates in detoxification organs (viscera), but can also penetrate critical neural tissues, potentially posing neurotoxic risks. Such tissue-specific accumulation is consistent with recent evidence showing that cephalopods may concentrate Hg in nervous system tissues even more than in muscle or digestive organs (Minet et al., 2021; Viana et al., 2021). A direct comparison between organs within the same locality (Zawia) indicates that brain tissue harbors higher Hg levels than viscera — for example, the maximum brain Hg (0.31 mg/kg) is nearly double the maximum in viscera (0.165 mg/kg). This supports the notion of selective tissue accumulation, possibly reflecting Hg affinity for protein-rich or lipid-rich

tissues such as neural tissues, which may allow for biomagnification within the organism (Seixas & Pierce, 2005; Viana et al., 2021). The observed spatial differences emphasize the critical role of environmental contamination sources in determining heavy metal bioaccumulation. The significantly elevated Hg in both viscera and brain of octopus from the Zawia area raises concerns regarding seafood safety and human exposure risk, especially for frequent consumers of cephalopods from industrialized coastal zones. In contrast, the lower Hg levels in samples from Marsa Zawagha-Sabratha-Sabratha suggest that octopus harvested from less polluted areas may pose a reduced risk. Although regulatory guidelines often focus on edible muscle tissue and may consider measured Hg concentrations (in this and prior studies) as within
health sciences and it publishes high quality original papers, theory-based empirical papers, review papers, case reports, conference reports/papers, technology reports, book reviews, commentaries, events and news

Conclusion

Mercury accumulates more in the brain than in the viscera within the same octopus's population. Octopuses from industrially impacted areas (Zawia Refinery) exhibit significantly higher Hg levels than those from relatively cleaner sites (Marsa Zawagha-Sabratha-Sabratha). Body size appears positively correlated with mercury accumulation. These findings emphasize the importance of both tissue-specific and regional assessments when evaluating seafood safety and the ecological impact of heavy metal contamination.

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