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Heavy metals distribution in the body parts of the cephalopods (*Sepia officinalis* and *Octopus vulgaris*) collected from the Mediterranean Sea, Egypt

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ABSTRACT

The present study aimed to evaluate the level of some heavy metals in two edible cephalopods; *Sepia officinalis* and *Octopus vulgaris* and investigate the distribution and accumulation of these metals in different body parts of cephalopods. The concentrations of seven essential and non-essential heavy metals (Mn, Fe, Cu, Ni, Pb, Zn, and Cd) were measured in the body parts (Head, Arms, Mantles, Digestive gland, Ink, and Viscera) of the investigated cephalopods which collected from three sites at Alexandria city from the Mediterranean Sea-Egypt during March 2017. The digestive gland of both species exhibited a similar pattern of some heavy-metal accumulation where it was the major part of the highest concentrations of Cu, Zn, and Cd. The highest concentration of Mn was found in the viscera of both studied species while the highest concentration of Fe was recorded in the viscera of the Sepia and the digestive gland of the octopus. The metal pollution index (MPI) in Octopus (5.95) was slightly higher than recorded in Sepia (4.38). The lowest value of MPI was found in the head and the mantle of sepia while for the octopus the lowest value of MPI was found in their arms and mantle.

INTRODUCTION

Marine molluscans are an essential seafood source for human feeding consumption due to their high contents of proteins, vitamins, minerals, and carbohydrates (Ajaya Bhaskar 2002; Zlatanos *et al*., 2006; Abdel-Salam and El Benasy 2015). According to FAO (2004) the cephalopods represent important economic seafood for human consumption and contribute to 14% of the world fisheries. Despite this, consumption of molluscan organisms caused toxicological effects for humans because of their ability to accumulate trace metals in their body from the environment (Gupta and Singh 2011; Rabaoui, *et al*., 2013; Conti and Finoia, 2010). Metal bioaccumulation by aquatic organisms has been the subject of considerable interest in recent years because of serious concern that high levels of metals may have detrimental effects on marine organisms and create problems concerning their suitability as food for humans (Abdel-Salam, 2009).

Cephalopods are considered one of these molluscan groups whose consumption increased during the last decades (Boyle and Rodhouse 2006; FAO, 2007). As well as some cephalopods accumulate essential and non-essential metals, which act as significant provenance of contaminants for their predators (Dorneles *et al*., 2007). Indeed, to the short life span, cephalopods are considered a dynamic biomonitoring species for pollution in the marine environment (Kojadinovic *et al*., 2011). Thus, cephalopods play an essential role in transferring metals not only to humans but also to their top predators (fishes, birds, and mammals) across the aquatic food chains (Amiard *et al.*, 2008). These metals were accumulated mainly in their digestive glands (Bustamante *et al*., 2002a,b; Raimundo *et al*. 2004; Napoleão *et al*., 2005; Pierrea *et al*., 2009; Raimundo and Vale 2008). Also, it is stated to be in some other parts/tissues (Gerpe *et al*., 2000; Villanueva and Bustamante, 2006; Napoleão *et al*., 2005). The naturally occurring trace metals have been reported to be of great importance in cephalopods as they are playing a key role in the activity of enzyme activations during ink formation, detoxification, and excretion (Derby, 2014).

Unfortunately, the coastal zone of the Egyptian coast in the South-eastern Mediterranean Sea especially at Alexandria city receives a huge amount of heavy metal pollution produced from anthropogenic activities like industrial waste activities, oil transportation. In addition to untreated sewage and wastewaters via the local-sewerage system and pouring into the sea (El-Nemr *et al*., 2007, Masoud *et al*., 2010). Therefore, many studies have been focused on heavy metals pollution in Alexandria coast (Khaled *et al*., 2010, Abdel Ghani *et al*., 2013, Okbah *et al.*, 2014; Soliman, 2015). The main objectives of this study were to evaluate the level of some heavy metals in two edible cephalopods; *Sepia officinalis* and *Octopus vulgaris* and investgates distribution and accumulation of these matals in different body parts of cephalopod.

MATERIALS AND METHODS

Sampling

Adult males of *O. vulgaris* and *S. officinalis* (100-150gm) were collected by net fishing from three sites (Abu Qir, Sedi- Besher and Al-Anfoshi) at Alexandria city from the Mediterranean Sea, Egypt, during March 2017. They were immediately iced and kept frozen at (-20°C) till heavy metals anaylses. Samples were transferred in an icebox to the laboratory at Sohag University. The ink sac, digestive gland, viscera, head, arm, and mantle tissues were dissected out, and stored at (- 20°C).

Preparation of samples and analytical procedure

From different parts of investgated species 1.5-2 gm samples were gentally dried in microwave oven at 105°C to avoid losses of volatile elements (Cd, and Pb). After cooling in the desiccators, dried samples were squashed in porcelain mortar to get a fine powder and stored at (- 20°C) till analysis (Khan, *et al*., 2013). For digestion, 0.5 gm of each sample was put into a Teflon vessel, 10 ml of HNO_3 (Sigma - Aldrich) were added and they were left overnight at room temperature. Afterward, they were cooled and diluted using deionized bi-distilled water to 50ml (Jinadasa, 2014). To determine metal concentrations, 1ml of each sample was applied to AAnalyst 4000 Atomic absorption spectrometer Perkin Elmer at Sohag University. Metal pollution index (MPI) was calculated to indicate the total metal load in the body parts of collected cephalopods by using the equation: $MPI = (M1xM2xM3x….Mn)^{1}/n$, Where, Mn is the concentration of metal (mg/g) in the body parts.

Data analysis

Data analysis was conducted with (IBM SPSS Statistics 22) and Excel (Office 2010). The paired sample t-test was used to test for differences in the means of heavy metals concentrations in the two studied cephalopods. The relationships between metals concentrations in different parts of sepia and octopus were assessed using the Pearson correlation. A probability value of $p < 0.05$ was considered statistically significant. Tables and graphs were plotted to determine variations in the levels of the studied heavy metals pollution within sites.

RESULTS

Table (1) presents the mean investigated heavy metal concentrations in different body parts of *Sepia officinalis* and *Octopus vulgaris* collected from three different sites in Alexandria coastal waters. For all body parts, *O. vulgaris* recorded a higher concentration of studied heavy metals than *S. officinalis*. These differences were statistically significant in case of Cu concentrations in mantel, digestive gland and viscera (t= 2.28, 4.128 and 2.679, p= 0.037, 0.001 and 0.021, respectively), Zn concentrations in ink sac and viscera (t= 2.539 and 3.67, p= 0.034and 0.002, respectively) and Fe concentration in digestive gland (t= 2.572, p=0.02).

The different body parts of *Sepia* and *Octopus* exhibited variation in the accumulation of heavy metals under study. Generally, Cu, Zn, and Cd in the digestive gland showed the highest metal concentrations compared with other sepia and octopus parts, while Mn concentration in viscera of sepia and octopus, Fe in viscera of sepia, Ni in the head of the sepia, and Pb in ink sac of octopus were higher than in the digestive gland and the other parts. For both species, the variations among body parts in Cu and Zn concentrations were statistically significant, where digestive glands recorded the highest

Table 1: Heavy metal concentrations (mean± SD) (ppm wet) in different body parts of *S. officinalis* and *O. vulgaris* collected from Alexandria coastal waters.

	Body Parts	Mn			Fe			Cu			Ni			Pb			Zn			Cd		
		Mean	±	Std.D	Mean	±	Std.D	Mean	±	Std.D	Mean	±	Std.D	Mean	±	Std. D	Mean	±	Std.D	Mean	±	Std.D
Sepia	Head	1.03	±	1.54	13.96		8.75	13.00	±	8.63**c**	5.73	±	6.81	1.25	±	1.03	14.29	±	4.39**c**	1.37	±	1.00
	Arms	2.23	±	4.14	13.33		8.97	8.41	±	8.47**d**	2.60	±	3.28	2.77	±	2.24	12.86	±	8.71**c**	1.97	±	1.96
	Mantle	0.79	±	0.90	14.64		9.41	7.93	±	5.80**d**	4.91	±	7.20	2.81	±	2.69	13.39	±	7.32**c**	1.52	±	1.65
	DG	1.10	±	0.82	38.03		28.32	80.34	±	66.15**a**	3.36	±	3.05	3.84	±	3.29	85.16	±	80.39**a**	2.08	±	2.09
	Ink	2.37	±	4.78	26.67		23.58	10.99	±	9.26**c**	3.60	±	4.23	2.99	±	3.21	11.70	±	6.91**c**	1.62	±	1.68
	Viscera	2.98	±	4.23	90.08		144.35	21.60	±	12.62**b**	4.18	±	3.78	3.40	±	4.97	18.66	±	7.96**b**	1.82	±	1.76
	F	0.872			2.121			9.184			1.087			0.933			7.248			0.365		
	***P* value**	0.439			0.179			**0.014**			0.364			0.470			**0.025**			0.734		
Octopus	Head	1.78	±	1.68**abc**	25.71		25.56**c**	16.78	±	10.91**c**	5.54	±	3.76	2.24	±	1.58	18.90	±	25.99**cd**	1.34	±	1.38
	Arms	0.58	±	0.66**dc**	13.60		7.05**c**	4.60	±	4.07**d**	6.62	±	6.25	3.84	±	3.63	9.27	±	3.36**d**	1.10	±	1.18
	Mantle	1.43	±	1.44**abc**	22.72		12.78**c**	18.17	±	12.15**c**	5.52	±	4.25	2.16	±	1.78	12.67	±	4.50**cd**	0.74	±	0.76
	DG	0.97	±	0.96**c**	81.89		42.61**a**	235.58	±	91.38**a**	7.52	±	5.37	2.72	±	2.70	91.98	±	33.05**a**	3.28	±	3.51
	Ink	0.67	±	0.89**bcd**	47.91		27.54**b**	51.70	±	67.58**b**	4.34	±	3.64	6.45	±	6.97	76.11	±	75.79**ab**	1.63	±	2.33
	Viscera	1.99	±	1.05**a**	37.87		26.39**bc**	47.62	±	26.26**b**	6.46	±	4.07	4.14	±	2.89	35.08	±	10.81**bc**	0.97	±	0.75
	F	3.499			13.260			34.352			1.480			2.875			13.314			2.489		
	***P* value**	**0.010**			**<0.001**			**<0.001**			0.250			0.096			**0.007**			0.115		

concentration of Cu (80.34 and 235.58 ppm for Sepia and Octopus, respectively) and Zn (85.16 and 76.11 ppm for Sepia and Octopus, respectively) in both species (Table 1). Also, *O. vulgaris* showed a significant difference among the body parts in accumulation of the metals; Mn (F= 3.499, P = 0.010), Fe (F= 13.260, P = 0.001). The highest concentration of Mn (1.99 ppm) was in the viscera while the highest concentration of Fe (81.89 ppm) was in the digestive gland.

Sepia officinalis stores metals mainly in the digestive gland. The levels of Cu (80.34 ppm), Pb (3.84 ppm), Zn (85.15 ppm), and Cd (2.08 ppm) in *S. officinalis* digestive gland were higher than other body parts. However, Mn (2.98 ppm) and Fe (90.08 ppm) recorded the highest accumulation value in Sepia's viscera (Fig. 1A). On the other hand, the accumulation of heavy metals in the different parts of *Octopus vulgaris* revealed that the highest concentration of Cu (235.58 ppm) followed by Zn (91.98 ppm), Fe (81.89 ppm), Ni (7.52), and finally Cd (3.28 ppm) was in their digestive gland. But, the levels of Mn (1.98 ppm) and Pb (6.45 ppm) in the viscera and ink sac of *O. vulgaris*, recpivtivly, were higher than other parts of the body (Fig. 1B).

Investigated heavy metals concentrations as well as metal pollution index (MPI) of different body parts of studied cephalopods indicated no significant differences between the collected sites (Table 2). Also, there was no difference between investigated species in MPI for all body parts, while for both species there were significant differences between body parts in MPI (Table 3). The digestive gland and Viscera recorded higher

MPI than other body parts for the two study species. The lower MPI of S. officinalis was recorded in head and mantle, while O. vulgaris recorded the lower MPI for arm and mantle (Fig. 2).

Table 2. Mean ± SD of metal pollution index (MPI) for different body parts of Cephalopods; *S. officinalis* and *O. vulgaris* collected from three investigated sites at Alexandria coast.

	Parts	Site1			Site2			Site3			F	*P* value
		Mean	±	Std.D	Mean	±	Std.D	Mean	±	Std.D		
Sepia	Head	3.00	±	2.66	2.19	±	1.93	2.70	±	0.95	0.129	0.882
	Arms	3.87	±	2.02	3.62	±	3.25	3.04	±	2.86	0.071	0.933
	Mantle	3.46	±	1.38	1.89	±	2.35	3.33	±	1.78	0.643	0.558
	DG	5.62	±	4.89	6.29	±	2.52	11.01	±	5.81	1.212	0.361
	Ink	3.45	±	3.16	4.14	±	3.17	3.77	±	2.18	0.043	0.958
	Viscera	9.49	±	6.49	3.63	±	1.00	4.27	±	1.68	2.024	0.213
Octopus	Head	3.78	±	2.07	5.46	±	2.75	5.05	±	4.50	0.217	0.811
	Arms	3.50	±	1.79	2.13	±	3.68	2.24	±	2.91	0.208	0.818
	Mantle	5.41	±	1.80	3.18	±	2.26	2.69	±	2.38	1.350	0.328
	DG	11.58	±	5.04	9.36	±	11.13	12.41	±	7.09	0.112	0.896
	Ink	4.49	±	5.21	8.25	±	0.53	5.99	±	3.35	0.832	0.480
	Viscera	7.66	±	1.38	7.66	±	2.40	6.20	±	2.01	0.545	0.606

Table 3. The difference between investgated species (Sepia: *S. officinalis* and Octopus *O. vulgaris*) in metal pollution index(MPI) for different body parts.

Parts	Sepia			Octopus			t	*P* value
	Mean	±	Std.D	Mean	±	Std.D		
Head	2.63	±	1.75	4.77	±	2.93	-1.87	0.08
Arms	3.51	±	2.41	2.62	±	2.60	0.75	0.46
Mantle	2.90	±	1.79	3.76	±	2.25	-0.90	0.38
DG	7.64	±	4.74	11.11	±	7.19	-1.21	0.24
Ink	3.79	±	2.51	6.24	±	3.51	-1.71	0.11
Viscera	5.80	±	4.39	7.17	±	1.86	-0.87	0.40
F	6.421			10.182				
P value	0.005			0.002				

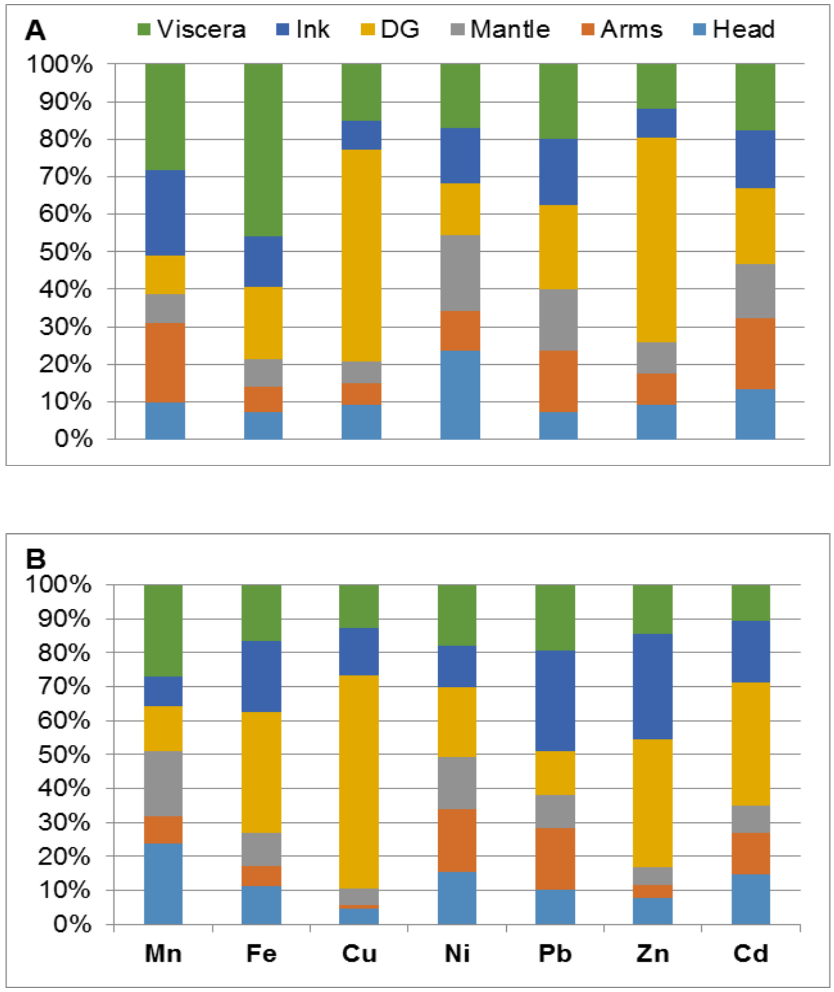

Fig. 1. Percentages of heavy metal concentrations in animal body parts; **A**: *Sepia officinalis*, **B**: *Octopus vulgaris*

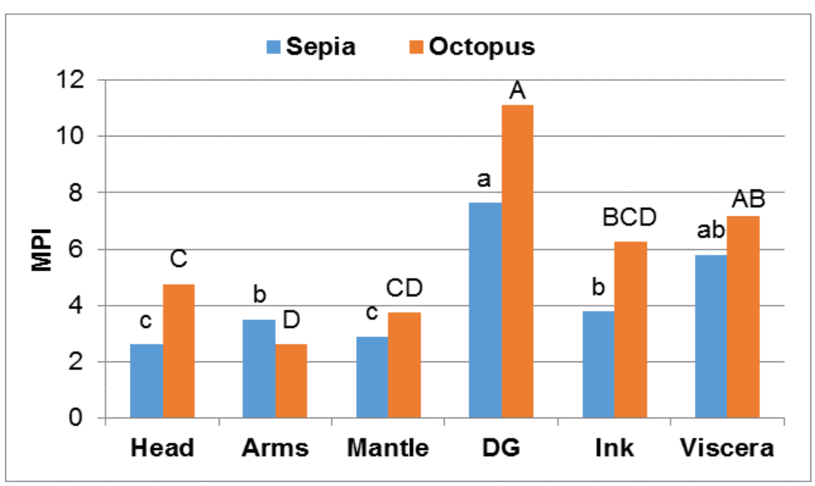

Fig. 2. Mean of metal pollution index (MPI) for deferent body parts of *Sepia officinalis* and *Octopus vulgaris*.

DISCUSSION

The present study showed that the highest concentrations of Cu, Zn, and Cd were recorded in the digestive gland of both studied cephalopods; *S. officinalis* and *O. vulgaris* from three sites in Alexandria. Roldán-Wong *et al.* (2018) reported that digestive glands are capable to accumulate higher levels of Cd than muscle. Pereira *et al.* (2009) stated that extremely high concentrations of Zn, Cu, Fe, and Cd were found in the digestive gland of *S. officinalis* and *Octopus vulgaris* from two coastal lagoons in Portugal. Cephalopoda digestive gland is considered the main part of metal detoxification and bioaccumulation. (Kadar *et al*., 2006; Kadar, 2007). Bustamante *et al*., (2002a) reported that all digestive gland tested of octopus displayed the highest Cd and Pb concentration, approving the primary role of this district in the bioaccumulation and detoxification processes of Cd and Pb and confirming the attendance of these metals at both sampling zones.

The current results are compatible with Bustamante *et al*. (1998) who emphasized cephalopods' ability to accumulate Zn, Cu, and Cd in their digestive glands, even in low metal contamination environments. Miramand and Bentley (1992) concluded that the digestive gland of *S. officinalis* is the major place of concentration for Ag, Cd, Co, and Zn. Monterroso, (2005) reported that the higher accumulation of Cd in the digestive gland of *S. officinalis* reflects higher Cd exposure. Rodrigo and Costa (2017) stated that the accumulation of toxicants in the digestive gland depends on their mechanisms of apical entry. Bustamante *et al*. (2002b) revealed that Cd and Zn enter the cephalopods' digestive gland directly via food and indirectly via blood, in the latter case if uptake occurs from seawater. Costa *et al*. (2014) concluded that, unlike bivalves, the cephalopod digestive gland possesses a complex network of arteriole-like blood vessels.

The present study had compared the metal levels found in the two studied cephalopod species samples and the legal categories for each metal according to the European Commission (Regulation EC No. 1881/2006 amended by EC No. 629/2008 and EC No. 420/2011). According to the regulation mentioned above, lead and cadmium concentrations in the sepia digestive gland (nonedible) in the present study were (3.84 mg/kg) and (2.08 mg/kg) respectively exceeded the maximum limits of heavy metals in cephalopods (1.0 mg/kg). Likewise, the concentration of Pb and Cd in the octopus's digestive gland were (2.72 mg/kg) and (3.28 mg/kg), respectively exceed the European Regulation of metals in cephalopods (1.0 mg/kg). Mshana and Sekadende (2014) concluded that the feeding habits of the octopus may also contribute to the elevated concentration of lead in the muscles and liver. According to FAO/WHO food standards programme Codex committee on contaminants in foods, 2018, the Maximum Level (ML) of Cadmium in cephalopods (applies to cuttlefishes, octopuses, and squids without viscera) was 2 mg/kg. The ML of Pb in fish (after removing the digestive tract) according to FAO/WHO, (2018) was (3 mg/kg).

The highest concentration of Mn was recorded in the viscera of both studied Cephalopods while the highest concentration of Fe was in the viscera of Sepia and the digestive gland of octopus. Duysak *et al*. (2013) concluded that the relationship between metal accumulation and cephalopod parts might be due to the difference in metabolic activities, various factors such as season, length and weight, and water's physical and chemical status.

There were no differences found between the two studied cephalopods in MPI but differences were recorded in different parts of their bodies. The highest value of MPI in the two studied cephalopods; was recorded in their digestive gland *S. officinalis* and *O. vulgaris* which are represented by (7.64) and (11.11), respectively. The MPI in Octopus (5.95) was slightly higher than recorded in Sepia (4.38). These results were less than reported by Ahdy *et al*. (2007), where MPI was (7.62) in Sepia and (9.55) in Octopus. The lowest value of MPI was found in the head and the mantle of sepia while for the octopus the lowest value of MPI was found in their arms and mantle.

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