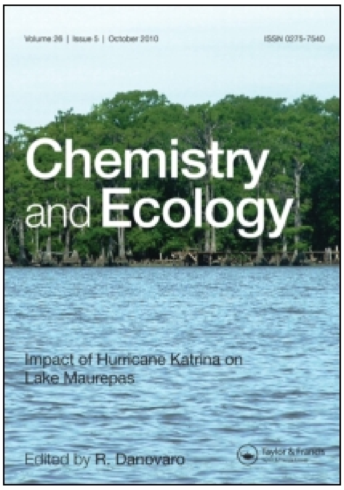

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Bioaccumulation of heavy metals in *Octopus vulgaris* from coastal waters of Alexandria (Eastern Mediterranean)

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Note

BIOACCUMULATION OF HEAVY METALS IN *Octopus vulgaris* FROM COASTAL WATERS OF ALEXANDRIA (EASTERN MEDITERRANEAN)

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Heavy metal concentrations (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) were determined in various tissues (hepatopancreas, branchial hearts, salivary gland, gills, genital tract, mantle, arms and skin) of *Octopus vulgaris* collected from three different contaminated sites in front of Alexandria (Egypt) during 2000. All collected tissues displayed high enrichment factors when compared to ambient levels. Heavy metal concentrations in most tissues displayed significant differences among sites, sizes and sex. This study suggests that hepatopancreas, and to a lesser extent branchial hearts, are better indicators of chronic Cu, Fe, Zn and Cd contamination than edible tissues. The enrichment factor (EF) for heavy metals in the hepatopancreas and in edible tissues allowed discriminating our samples into three main groups; (1) $EF > 55$ (Cu), (2) EF ranging from 15 to 7.5 (Fe, Cd and Zn) and (3) $EF < 2.5$ (Mn, Co, Pb, Ni and Cr). In the Mex area, the most polluted site, the highest bioaccumulation of Cu, Fe, Zn and Mn was observed. In the Kayet Bey were observed high levels of Pb and in Agami high concentrations of Cd were recorded. Females bioaccumulated Pb and Fe and less Zn, Cr, and Co than males, whereas Ni and Mn concentrations were sex independent. Concentrations of both Mn and Co in gonads, Cr in branchial hearts and Zn in both gills and mantle increased linearly with animal weight. Conversely, concentrations of Cd and Ni in gonads, Mn in mantle and Pb in hepatopancreas were inversely related with body size. Finally, heavy metal bioaccumulations in the liver were independent from animal size.

Keywords: Bioaccumulation; Heavy metals; *Octopus vulgaris*; Eastern Mediterranean

1 INTRODUCTION

Octopus vulgaris is the most abundant species of the order Octopoda: (Cephalopoda), of the Mediterranean Sea. The species has a worldwide distribution in the coastal water down to 200-m depth in tropical and subtropical ecosystems. *Octopus* is abundant also in the East Atlantic Ocean, in the Turkish waters, in the Atlantic from Long Island to southern Brazil, from Southern North Sea to the Cape of Good Hope and is common in the Gulf of Mexico and Caribbean Sea (Nesis, 1987; Roper *et al.*, 1984; Katagan and Kocatas, 1990). In the Mediterranean coastal waters of Alexandria this species is common at shallow depths and has a important economical relevance (Abdalla, 2000).

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Various investigators have studied the accumulation of metals by Cephalopoda (Rocca, 1969; Bryan, 1984; Miramanda and Bentley, 1992). Most studies dealt with the digestive glands of squids and *Octopus* species, and have principally examined metabolized elements such as copper, iron and zinc (Ghiretti-Magaldi *et al.*, 1958; Nardi *et al.*, 1971).

Some data have been reported for Cd, Ag, Cu and Hg (Renzoni *et al.*, 1973, along Tyrrhenian coasts), for squid livers (Martin and Flegal, 1975) and for the digestive gland of squids (Smith *et al.*, 1984). Other authors have investigated the branchial heart of *Octopus vulgaris*, reporting that this organ might contain high iron concentrations (Ghiretti-Magaldi *et al.*, 1958; Nardi and Steinberg, 1974) and concentrate some other elements, such as Cobalt (Ueda *et al.*, 1979), Vanadium (Miramand and Guary, 1980), Americium and Plutonium (Guary *et al.*, 1981). The accumulation of metals by such organisms potentially depends on uptake from food as well as from the water medium. Several studies suggest that food and particulate matter are much more important sources of metals than water (Hoss, 1964; Pentreath, 1973a, b; Davies *et al.*, 1981; Dallinger *et al.*, 1987). In most of the Egyptian Mediterranean waters, this *Octopus* species feeds on benthic fishes, cephalopods food items represented by arms, tentacles, suckers, flesh and beaks. Also crustacean food items are largely represented by chitin's shells, legs and flesh of crabs or shrimps (Abdalla, 2000).

Little information is available yet on the bio-accumulation of heavy metals in the tissues of cephalopods (Bryan, 1984). Concentrations of Pb, Cu, Zn, Fe, Cd and Mn have been investigated by Ghazaly (1988) in the whole body of *Octopus vulgaris* from Alexandria coastal water. Such studies are important to evaluate the contamination level in the edible animal tissues and internal organs. The present study focused on the level of heavy metals in edible animal tissues in relation to relevant public health standards recommended by different councils such as the Australian National Health and Medical Research Council (NHMRC) and subsequently adopted by the Australian food standards council (Rayment, 1991).

2 MATERIALS AND METHODS

Twelve individuals of *Octopus vulgaris* of which five females (200–460 g) and seven males (200–600 g) were used. Four animals were caught from the coastal area of Alexandria at each of Kayet Bay, Mex and Agami. Different amounts of effluent wastes are discharged into the coastal area of Alexandria, inside the Eastern harbour, eleven small sewers discharged an amount of 15,000 cubic meters daily (Anon, 1978) and more than 0.5 million cubic meters daily of domestic water discharge throughout Kayet Bay pipelines. The Western harbour area receives about 6 million cubic meters daily of untreated industrial, drainage and domestic waters. Mex area is subjected to several sources of wastes, 7.7 million cubic meters daily are discharged through Ummum drain from the neighbouring lake Maryut (El-Sayed *et al.*, 1988). Immediately after thawing, the samples were dissected and the gut contents removed. Length, weight and sex of all specimens were analyzed. All samples were deep-frozen in the laboratory a few hours after capture. To detect any eventual variability in element concentrations in relation to the sex, genital tracts of males and females were analyzed separately. For each individual, the liver part of hepatopancreas, free of vessels and capsules, the salivary glands, gills and branchial hearts as well as suitable weights of skin, arms and mantle were taken. All each of these tissues was dried at 100 °C until constant weight and then ground. The homogenized powder was digested with 5 ml-concentrated HNO_3 per 0.3–0.4 g of dried sample and the Cu, Zn, Mn, Fe, Cr, Cd, Co, Ni, and Pb concentrations were analyzed using Atomic Absorption Spectrophotometer AAS.

3 RESULTS AND DISCUSSION

3.1 Metals in Individual Tissues

The concentration of nine metals (μg/g-wet wt.) in different tissues of *Octopus vulgaris* from three sites in front of Alexandria are compared in Table I. The present investigation displayed the presence of different groups characterized by different accumulation rates for various elements in different tissues. In spite of the small weight of the hepatopancreas liver compared to the whole animal (<5%), metal concentrations in this organ always exceed those in the animal mantle, arms or skin. Sometimes this organ contained a Cu concentration 50 times higher than other tissues (high accumulation) whereas Fe, Cd and Zn displayed a moderate accumulation and other elements only a low accumulation rate. This finding is in agreement with Ghiretti and Violante (1964) and Rocca (1969) who found >60% of the whole animal copper in hepatopancreas. This is probably due to storage of the metabolizable elements in the digestive gland. Tanaka *et al.*, (1983) and Finger and Smith (1987) showed that Cd accumulation was associated with high molecular weight material in the digestive gland of squids. Ueda *et al.*, (1979) reported 3.52 μg/g wet wt. of Co in *Octopus vulgaris* liver, which is within the range of our average (3.59 μg/g wet wt.).

Concentrations of heavy metals determining in the liver of *Octopus vulgaris* followed the order: Cu > Fe > Zn > Cd > Ni > Pb > Cr > Co > Mn.

TABLE I Heavy metal concentrations (ppm wet wt) in tissues of *Octopus vulgaris* collected from Alexandria coastal waters. Values are means of four samples.

Location	*Metal*	*Mantle*	*Liver*	*Gonads*	*Salivary gland*	*Branchial heart*	*Gill*	*Skin*
Mex		1.81	9.18	1.01	4.34	3.25	1.22	1.79
Kayet Bey	Cd	1.30	20.03	1.77	3.05	2.48	1.79	1.43
Agami		1.40	36.16	2.56	4.43	3.79	1.98	2.31
Mex		1.75	2.72	3.00	1.29	3.46	1.73	1.67
Kayet Bey	Co	1.77	6.10	3.20	1.38	5.43	1.91	2.67
Agami		1.96	1.95	0.66	3.01	2.49	0.87	1.13
Mex		5.89	6.88	7.35	4.98	7.35	3.08	8.33
Kayet Bey	Cr	5.98	9.30	6.83	8.60	9.58	4.83	3.35
Agami		5.98	10.98	10.03	8.18	6.18	2.78	6.03
Mex		12.63	390.00	24.1	116.30	121.1	38.30	16.33
Kayet Bey	Cu	6.84	715.00	8.15	56.28	57.50	23.90	12.50
Agami		5.24	632.00	26.30	55.50	77.55	27.75	10.85
Mex		15.68	138.50	27.63	31.90	69.30	15.85	18.03
Kayet Bey	Fe	10.56	171.30	18.30	18.50	40.88	11.88	11.25
Agami		12.20	179.70	21.23	27.10	60.45	10.80	19.10
Mex		1.48	2.97	2.42	1.54	2.88	0.10	2.96
Kayet Bey	Mn	1.10	0.64	1.41	0.10	0.97	1.19	1.44
Agami		1.31	4.02	0.43	0.18	1.79	0.10	0.12
Mex		8.67	8.86	15.81	18.10	34.20	8.45	4.61
Kayet Bey	Ni	11.64	13.00	12.1	16.28	74.4	5.5	6.3
Agami		9.16	20.5	17.95	8.58	51.1	8.43	13.80
Mex		1.84	5.65	1.51	2.04	8.84	2.14	3.62
Kayet Bey	Pb	7.11	12.4	14.45	22.48	9.41	4.42	7.35
Agami		7.40	16.49	7.24	5.83	8.27	3.08	6.63
Mex		19.95	111.6	42.13	35.58	58.4	20.98	18.1
Kayet Bey	Zn	15.46	109.4	26.45	34.65	13.80	12.73	11.95
Agami		13.29	142.4	27.6	11.80	22.85	10.45	15.03

It is forth noting that the higher content in Cu and Fe in the branchial heart than in other tissues appears to be due to the presence of haemocyanin and polyhedral cells containing granules with purple brown pigments (adenochromes) which are rich in Fe (Fox and Updegraff, 1943; Ghiretti-Magaldi *et al.*, 1958; Nardi and Steinberg, 1974; Schipp and Hevert, 1978). Other studies have also shown that these organs concentrate Co (Ueda *et al.*, 1979, Nakahara *et al.*, 1979) but do not concentrate Cd or Zn (Miramanda and Guary, 1980). For these three elements, our data can not be compared. We report here that Cu, Fe, Ni and Zn were concentrated in the branchial hearts of *Octopus vulgaris* (up to 90 ppm) while Pb, Cr, Co, Cd and Mn did not exceed 10 ppm.

The concentrations of elements in branchial heart followed the order: Cu > Fe > Ni > Zn > Cr > Cd > P > Co > Mn. Our data reflect lower level of Cu in the branchial hearts than those measured by Miramanda and Guary (1980) in *Octopus vulgaris* of the Mediterranean Sea. The same pattern is observed for Co and Fe, which are concentrated in the branchial hearts at a level of 47 $\mu g\,g^{-1}$ dry wt. (Co) and 650 $\mu g\,g^{-1}$ dry wt. for Fe (Ueda *et al.*, 1979).

The salivary glands concentrate Cu and to a less extent Fe, Ni and Zn while gills do not seem to concentrate any element (except Cu). Heavy metals bioaccumulations in glands followed the order: Cu > Zn > Fe > Ni > Pb > Cr > Cd > Co > Mn.

It is interesting that the arrangements of metal concentration in liver, branchial hearts and salivary glands were similar for the highest Cu element or the lowest two, Co and Mn. Conversely, heavy metal accumulation in the skin was rarely found during this work.

Octopus vulgaris specimens are non-migratory and would therefore presumably represent an useful indicator of heavy metal contamination in the marine environment (Boletzky, 1989; Boucaud-Camou and Boismery, 1991).

3.2 Comparison among sites with different pollution sources

Differences in heavy metal concentrations between the three sites were more pronounced than differences between male and female. The edible tissues (arms and mantle) as well as internal organs (except for liver) in specimens from Mex, the most polluted site, exhibited the highest metal concentration. This was evident for Fe, Cu, Zn and Mn and to a lesser extent for Ni and Cd. In Kayet Bay, which received considerable amounts of sewage wastewater, levels of Pb were particularly high while in Agami specimens showed highest concentrations of Cd. Unexpectedly high levels of heavy metal concentration (except Co) were found in the hepatopancreas liver of Agami specimens, the least polluted area whereas low concentration were found in Mex, the highest polluted site.

3.3 Effect of size on metal concentration

The relationship between size and heavy metal bioaccumulation was variable. The percentage of dry weight to wet weight (dry matter) of the mantle was found to slightly increase from 16.9% in the small size animals (200 g) to 18.3% in the largest specimens (600 g). Skin showed an inverse relationship with body size, while other tissues did not display a regular pattern. The concentration of heavy metals in different tissues of *O. vulgaris* either decreased with the increasing body size (*e.g.* Cd and Ni in gonads), Mn in mantle and Pb in liver, or increases with size (Mn and Co) in gonads, Cr in branchial hearts and Zn in mantle and gills. Highest levels also observed in the liver of animal of intermediate sizes (300 g). It is interesting to note that the concentrations of most of the studied elements were negatively correlated with the body size, particularly in the edible tissues; $r = -0.34$ for Fe, -0.33 for Cr, -0.32 for Mn, -0.25 for Cd, and -0.20 for Cu ($n = 24$).The largest sizes exhibited lowest metal concentrations in mantle (M) and arms (A; Tab. II). The same relationship could

TABLE II Mean concentrations of heavy metals (mg/kg wet wt) in the edible tissues (Arms + Mantle) of *Octopus vulgaris* in relevant to location, sex and animal weight.

		Location			*Sex*			*Animal weight (g)*				
Metal	*Agami*	*Mex Bay*	*Kayet Bay*	*Mean*	*Male*	*Female*	*Mean*	*200*	*300*	*400*	*600*	*Mean*
Cd	1.4	1.9	1.2	1.5	1.4	1.7	1.6	1.5	1.3	1.6	1.9	1.6
Co	5.0	1.8	1.8	2.9	2.2	1.3	1.8	1.7	2.3	2.2	2.0	2.1
Cr	6.0	5.9	6.0	6.0	6.3	5.2	5.8	5.6	6.5	8.2	3.9	6.1
Cu	5.3	12.6	6.9	8.3	8.3	14.4	11.4	4.7	5.8	13.2	7.0	7.7
Fe	12.2	15.7	10.5	12.8	12.1	14.6	13.4	13.5	11.0	9.9	11.8	11.6
Mn	2.3	1.5	1.1	1.6	1.6	0.7	1.2	1.8	1.6	1.0	0.4	1.2
Ni	9.2	8.7	11.7	9.9	10.0	7.0	8.5	10.6	7.7	16.2	7.9	10.6
Pb	7.5	1.9	7.1	5.5	4.5	4.8	4.7	6.6	9.9	7.6	10.9	8.8
Zn	13.3	20.0	15.5	16.3	16.7	15.7	16.2	10.2	14.6	13.6	18.0	14.1
Total	62.0	70.0	61.8	64.8	63.1	67.4	65.3	56.2	60.7	73.5	63.8	63.6

also be observed in internal organs such as gonads and branchial hearts for Fe, in gonads for Cu and in branchial hearts and salivary glands for Cd. The largest specimens, however, bioaccumulated much more Cu in their skin, salivary glands and gills whereas Pb was accumulated in arms and gonads, Cr in skin and Co in gonads.

3.4 Sex and heavy metal bioaccumulation and safe levels

As previously mentioned, the hepatopancreas liver of both sexes is the major organ for metal bioaccumulation of Cu, Fe, Zn, and Cd and to a lesser extent of Mn and Pb. *O. vulgaris* females accumulated in edible tissues preferentially Pb and Fe and less Zn, Cr and Co than males. In the female livers, the concentration of Fe, Cu, Ni, Pb and Cd were also higher than those of males. Miller *et al.*, (1992) found a large difference between sexes in the concentration of Zn in gonad tissues. The female edible tissues contain Cu and Pb twice as much than in males while concentrate Mn at low ratio 0.3 or 0.5.

We investigated the variability in heavy metal concentrations in mantle and arms of *Octopus vulgaris*. *Octopus vulgaris* was specimens collected in coastal waters of Alexandria as a function of tissues, site, size and sex. To do this we summed up concentrations of all heavy metals determined and referred to one kilogram of edible tissues. Data in Table II report the metal content in one kilogram of arms and mantles (A + M) of *Octopus vulgaris* males and females caught from the three sites in front of Alexandria. The potentially toxic heavy metals represented ca 40% of the total metal content.

The level of copper in mantle is nearly doubled high when compared with levels in arms. But this trend is completely reversed for lead and potentially toxic metal, with concentrations in the arms being ten times higher than mantle, especially in larger animals (600 g). The accumulation of other heavy metals in either arms or mantle did not display clear trend.

A limited spatial variability can be observed in the Mex area 70 μg of metals g^{-1} of tissue were found while in Agami or Kayet Bay ca 62 $\mu g\, g^{-1}$ were reported. However, toxic metal fraction displayed however unexpected levels in Agami (29 $\mu g\, g^{-1}$) and in Kayet Bay (27.8 $\mu g\, g^{-1}$) with values higher than in the Mex area (20.2 $\mu g\, g^{-1}$).

The edible fraction of tissues (A + M) of the small size specimens accumulated the lowest metal content (56 $\mu g\, g^{-1}$ wet wt). It increases with size up to reach 73.5 $\mu g\, g^{-1}$ in specimens of 400 g weight and decreased again to 63.8 $\mu g\, g^{-1}$ in 600 g individuals. It is interesting to notice that the potential toxic elements content in either the smallest or the largest animals are nearly identical and the variation is only detectable for metals having potential role in metabolic activities. Although females of *O. vulgaris* contained higher metal content than males (67.3 and 63.1 $\mu g\, g^{-1}$), respectively, the content of potential toxic metals in the female is slightly lower if compared with that in male, 2.20 and 2.44 $\mu g\, g^{-1}$, respectively.

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