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Subcellular distribution of Ag, Cd, Co, Cu, Fe, Mn, Pb and Zn in the digestive gland of the common cuttlefish *Sepia officinalis*

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Running title: Cd, Co and Cu were mainly associated to cytosolic compounds in the cuttlefish digestive gland.

ABSTRACT

The subcellular fractionation of the digestive gland cells of the common cuttlefish Sepia officinalis was performed in order to investigate the distribution of metals 1) between organelles and cytosol and 2) in the different cytosolic fractions separated by gel-filtration chromatography. Total metal concentrations vary over 3 orders of magnitudes, ranging from 2.2 $\mu\text{g}\cdot\text{g}^{-1}$ dwt for Pb to 612 $\mu\text{g}\cdot\text{g}^{-1}$ dwt for Zn. With the exception of Cd, Co and Cu, metals were mostly bound to the organelles. Whereas no specific organelle compartment was found for Mn, Pb and Zn, Fe was mainly associated to nucleus, brown body, and “boule” fraction (i.e. 52%) and 44% of the total Ag was contained in the lysosomal and mitochondria enriched fraction. The link of metals with hydrosoluble proteins in the cytosolic fraction was investigated at 254 and 280 nm. Direct relationship between cytosolic metal and metallothioneins could only be established for Ag and Cu whereas Cd and Zn seems to mainly bind high (>70kDa) and low (< 4kDa) molecular weight proteins.

Keywords: cephalopod; metal; trace element; detoxification; metallothionein; chromatography

INTRODUCTION

Exclusively marine organisms, cephalopods are active predators found from polar to tropical ecosystems and from the shallow waters to very deep ocean environments. As well, they constitute a major food source for many top predators species (see the reviews by Clarke 1996, Croxall & Prince 1996, Smale 1996, Klages 1996). They have therefore a key role in many marine ecosystems and are also of increasing interest for worldwide fisheries (Amaratunga 1983, Rodhouse 1989). Despite such ecological and economical importance, metal, radioactive and organic contaminants have been globally poorly studied in cephalopods.

Concerning metals, cephalopods concentrate several trace elements such as Ag, Cd, Cu or Zn at sometimes very high concentrations (e.g. Martin & Flegal 1975, Miramand & Bentley 1992, Bustamante et al 1998). Most of the studies reported the major role of the digestive gland in the bioaccumulation mechanisms, this organ suspected to have a key function in the metabolism of most metals in cephalopods (e.g. Miramand & Bentley 1992, Bustamante et al 2002a, 2004). Thus, the digestive gland of cephalopods constitutes the main storage organ for both essential (e.g. Co, Cu, Fe, and Zn) and non essential elements (e.g. Ag, Cd, Pb and V) independently of the considered species and of its area of origin (e.g. Miramand & Guary 1980, Smith et al 1984, Finger & Smith 1987, Miramand & Bentley 1992, Bustamante et al 1998, 2002a, 2004, Miramand et al 2006). Thus, around 70 to 98% of the whole body burden of metals could be readily stored in the digestive gland (Miramand & Bentley 1992, Bustamante et al 2002b). This could result from the very long half-life of some elements like Cd and Co (Bustamante et al 2002a, 2004) and/or to very efficient translocation processes from other organs and tissues to the digestive gland (e.g. Ag or Cs; Bustamante et al 2004, 2006). In the case of Cd and Co for example, the residence time of the metal in the whole organism could be longer than the life span of the cephalopods, meaning that almost all the

assimilated metal is definitively sequestered in the digestive gland (Bustamante et al 2002a, 2004). In contrast to Cd and Co, Ag displays a far faster turn-over in cephalopods (Bustamante et al 2004). Even the dissolved pathway is probably the main route for Ag accumulation in cephalopods, the digestive gland contains most of the whole body burdens of this metal (Miramand and Bentley 1992, Bustamante et al 2000, 2004). This suggests that very efficient translocation processes allow the transfer of Ag from tissues and organs in contact with seawater to the digestive gland for detoxification purposes (Bustamante et al 2004).

In both cases, i.e. metals directly stored for a long time in the digestive gland and metals having a peculiar tropism to this organ, the elevated concentrations reported on metal bioaccumulation in cephalopods suppose the occurrence of efficient storage and detoxification mechanisms to counteract the toxicity of metals (e.g. Simkiss & Taylor 1982, Phillips & Rainbow 1989). Detoxification mechanisms of marine invertebrates mainly involve the precipitation or co-precipitation of metals on amorphous granules and the binding on proteins, which can be non-specific (e.g. transferrin, ferritin) or specific to one or more metals (e.g. vanabins, metallothioneins) (e.g. Durand et al 2002, Ueki et al 2003). One well known detoxification strategy involving proteins is the binding of some trace metals to metallothioneins (MTs) which play a role in the homeostasis of the essential metals, Cu and Zn, but are induced by various other metals (i.e. Ag, Cd, and Hg) (Engel & Brouwer 1989, Cosson et al 1991, George & Olsson 1994). Thus, MTs are considered to be involved in Ag, Cd and Hg detoxification (Dallinger 1993, 1995, Roesijadi 1992, 1996, Viarengo & Nott 1993). In cephalopods, association of MT with metals in the digestive gland seems to mainly concern Cu and, to a lesser extent, Cd and Zn (Tanaka et al 1983, Finger & Smith 1987). In the digestive gland, most of Cd and Zn appear to be bound to other cytosolic proteins than MTs (Tanaka et al 1983, Finger & Smith 1987, Castillo et al 1990).

Considering the elevated metal concentrations occurring naturally in the digestive gland of cephalopods, the aim of our study was 1) to investigate the metal distribution between the different organelles and the cytosol, 2) to provide insight on the implication of hydrophilic proteins such as MTs in the detoxification mechanisms. The common cuttlefish *Sepia officinalis* was selected as an experimental model, and the subcellular distribution of various metals, i.e. Ag, Cd, Co, Cu, Fe, Mn, Pb and Zn, were considered in male mature individuals. Finally, the cytosolic fraction obtained were chromatographed to determine the association of the different metals to the hydrophilic proteins.

MATERIALS & METHODS

Biological material

Male mature common cuttlefish were caught in the Bay of Seine (French coast of the English Channel) and kept alive at the most two days in outflow tanks. Animals belonging to the same age class, from the same sex and sexual maturity state were selected in order to minimise the biological variability (n=4, total weight 785 ± 84 g). Prior to the experimentation, each cuttlefish were anaesthetised in seawater containing 2% ethanol and rapidly dissected. The digestive gland was carefully removed, weighted (n=4, 37 ± 10 g) and prepared both for direct heavy metal analysis and for subcellular fractionation.

Subcellular fractionation

Aliquots ranging from 1 to 2 g of each individual digestive gland were homogenised with a mortar and pestle on ice with 4 volumes of a 20 mM TRIS-HCl, 0.25 M sucrose buffer (Tanaka et al 1983), at pH 8.6. The homogenates were successively centrifuged at 600 g for 10 min, 10 000 g for 10 min and 100 000 g for 60 min at 4°C in a Beckman LE-70 ultracentrifuge. This procedure giving 6 different fractions is summarised in Figure 1. Each

pellet was collected in order to determine the metal concentrations in 1) the membranes, nuclei, “boules” (which are considered as heterolysosomes and heterophagosomes involved in intracellular digestion of cephalopods (Boucaud-Camou 1976, Boucaud-Camou & Yim 1980)) and brown bodies fraction, 2) the mitochondrial and lysosomal fraction, 3) the microsomal fraction. The accuracy of the sequential separation was controlled by Transmission Electronic Microscopy after fixation in 4% glutaraldehyde and post-fixation with osmium tetroxyde in 0.4 M cacodylate buffer at pH 7.3. The particle-free supernatant fraction (cytosol) was removed for heavy metal analysis and for gel filtration chromatography.

Gel-filtration chromatography

Prior to the gel filtration chromatography, total proteins were quantified in the collected cytosol following Lowry et al (1951). Then, 2 ml of this fraction were chromatographed on a Sephadex G-75 superfine (16 X 800 mm) column (Pharmacia) equilibrated and eluted with 20 mM Tris-HCl buffer, pH 8.6 at 4°C, containing 50 mM NaCl and 3 mM NaN₃. The column was maintained at 4°C and the samples were collected as 4 ml fractions. The ultraviolet absorbance of the eluate was measured at 254 and 280 nm with a U-1100 Hitachi spectrophotometer. In each eluted fraction, the heavy metal concentrations were also determined. The column was calibrated for molecular weight estimations with Ovalbumine (43 kDa), Chymotrypsin (25 kDa), Ribonuclease (13.7 kDa) and Glucagon (3.5 kDa) as standard markers. We also used equine renal metallothionein (13.4 kDa, Kojima et al 1976) to identify the fractions containing MTs.

Metal analyses

Samples of the digestive gland were previously dried at 80 °C to constant weight prior to analysis. The dried digestive gland samples, the pellets and the particle free supernatant resulting from the subcellular separation and the different fractions separated by gel chromatography were digested with 5 ml of 14N ultrapur HNO₃ at 100°C during 3 days. After evaporation of the acid, the residues were taken up in 5 ml 0.3 N HNO₃ and analysed for Ag, Cd, Co, Cu, Fe, Mn, Pb, and Zn by flame and graphite furnace atomic absorption spectrophotometry with a Zeeman Hitachi model 180-70.

Quality control was assessed by heavy metal analyses in blanks and reference materials. Thus, Orchard–Leaves (National Bureau of Standards) and MA-A-2 fish flesh standard (IAEA) were treated and analysed in the same way as the samples. Our results for the standard reference materials were in good agreement with the certified values (Table 1). The detection limits were ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight): 0.004 (Cd), 0.02 (Ag), 0.1 (Co, Mn, Pb), 0.5 (Cu, V, and Zn) and 2.5 (Fe). Results are also expressed in micrograms per gram of the dry tissue weight ($\mu\text{g}\cdot\text{g}^{-1}$ dwt).

RESULTS

Metal concentrations

The concentrations of Ag, Cd, Co, Cu, Fe, Mn, Pb and Zn are shown in Figure 2. Among the analysed metals, Zn is the most concentrated, reaching up to 600 $\mu\text{g}\cdot\text{g}^{-1}$ dwt, followed by Fe ($424 \pm 142 \mu\text{g}\cdot\text{g}^{-1}$ dwt) and Cu ($362 \pm 114 \mu\text{g}\cdot\text{g}^{-1}$ dwt). All other elements displayed far lower concentrations, i.e. ranging from 2.2 $\mu\text{g}\cdot\text{g}^{-1}$ dwt for Pb to 13.6 $\mu\text{g}\cdot\text{g}^{-1}$ dwt for Cd.

Subcellular distribution

The partitioning of metals among the 1) nuclei and brown bodies, 2) lysosomes and mitochondria 3) microsomes and 4) soluble cytosolic fraction is presented in Table 2. Most of the Cd, Co and Cu were associated with hydrosoluble cytosolic compounds whereas Ag, Fe, Mn, Pb and Zn were mostly bound to the organelles. Fe is mainly associated to the nuclei and brown bodies (52%) and 44% of the total Ag is contained in the lysosomal and mitochondria enriched fraction. It is noteworthy that Co, Cu, Pb and Zn are equivalently distributed in each pellet (Table 2).

Metal associated to proteins

Chromatographic Sephadex G-75 elution profiles of the absorbance at 254 and 280 nm obtained for cytosol from the digestive gland of Sepia officinalis were used to determine the metal concentrations (mg.l^{-1}) in the collected fractions containing the proteins separated by their molecular weight (Figure 3). A first peak centred on fraction 11 corresponds to the void volume (macromolecules larger than 70 kDa, such as haemocyanin) and a second peak between fractions 40 and 44 indicates a particular richness in small proteins and polypeptides (4 kDa and less). MTs used for calibration fell in fraction 26 with an elevated 254/280 nm absorbance ratio (equal to 16). All metals were associated with high and low molecular weight proteins (> 70 kDa and lower than 4 kDa, respectively), except Ag which was not detectable in the small protein fraction. Intermediate proteins ranging from 10 to 20 kDa did not contain detectable amounts of Fe and Mn. Ag and Cu were the only metals showing a peak in the fraction 26 region among those expected to bind MTs (Ag, Cd, Cu and Zn). Cd and Zn displayed a very similar distribution with a major fraction associated to high molecular weight proteins for both metals.

DISCUSSION

The metal concentrations measured in the digestive gland of male adult cuttlefish from our study globally fell within the range of values reported for Sepia officinalis from the English Channel (Miramand & Bentley 1992), the Bay of Biscay (Schipp & Hevert 1978, Bustamante 1998, Bustamante et al 1998) and the Mediterranean (Bustamante et al 2002a). Although globally poorly documented, metal concentrations in cephalopods have received increasing interest as these molluscs play a major role both as predators and food items in marine ecosystems. The central role of the digestive gland in metal bioaccumulation has been highlighted many times, particularly for toxic metals such as Ag and Cd (e.g. Martin & Flegal 1975, Miramand & Bentley 1992, Bustamante et al 2002a, 2004) but the detoxification processes occurring in this organ remain poorly understood (Bustamante et al 2002b). Investigations on detoxification mechanisms have focused either on the subcellular distribution of metals between cytosol and organelles (Rocca 1969, Tanaka et al 1983, Finger & Smith 1987, Bustamante et al 2002b, Craig & Overnell 2003), the involvement of hydrosoluble proteins in the binding of metals (see also Declair et al 1978, Ueda et al 1985, Castillo et al 1990, Castillo & Maita 1991) or on the histochemical and microanalytical localisation of metals within the digestive gland cells (Schipp & Hevert 1978, Martoja & Marcaillou 1993). Moreover, the subcellular distribution of metals mainly concerned Cd, Cu and Zn (i.e. Rocca 1969, Declair et al 1978, Tanaka et al 1983, Ueda et al 1985, Finger & Smith 1987, Castillo et al 1990, Castillo & Maita 1991, Bustamante et al 2002b, Craig & Overnell 2003) but very limited information is available for other elements. Indeed, to the best of our knowledge, the studies of Tanaka et al (1983) and Finger & Smith (1987) are the only ones providing data on other metals, i.e. Ag and Fe, and ²¹⁰Po, respectively. The few studies on metal detoxification in cephalopods have considered various models, i.e. cuttlefishes (Declair et al. 1978, Schipp & Hevert 1978, Martoja & Marcaillou 1993,

Bustamante et al 2002b), squids (Tanaka et al 1983, Finger & Smith 1987, Castillo et al 1990, Castillo & Maita 1991, Bustamante et al 2002b) and octopuses (Rocca 1969, Ueda et al 1985, Bustamante et al 2002b). Consequently, results are often very different between authors and sometimes contradictory. Therefore, there is a need to provide more information on the subcellular distribution of heavy metals in general and on poorly or not yet studied elements also highly concentrated in the digestive gland of cephalopods. In this respect, our study provides the first insight about the subcellular distribution of Co, Mn and Pb in cephalopods.

Our study of metal distribution between the insoluble (membranes and organelles) and soluble (cytosol) fractions of the digestive gland leads to the conclusion that metals can be separated between those mainly associated to hydrosoluble compounds, i.e. Cd, Co and Cu, and those mainly associated to organelles, i.e. Ag, Fe, Mn, Pb and Zn. This shift does not therefore correspond to the essential or non essential character of the metals but rather to the result of specific regulation/detoxification processes.

As already mentioned, the scarce information on the subcellular distribution of trace elements in cephalopods put forward that 50 to 90 % of the Cd is usually found in the soluble fraction of the digestive gland of cephalopod from the field (Finger & Smith 1987, Castillo et al 1990, Bustamante et al 2002b), even if the squid Todarodes pacificus does not follow this trend with only 26 ± 3 % of the metal being present under a soluble form (Tanaka et al. 1983). Our results for S. officinalis are consistent with this general trend (Table 2), suggesting the presence of mechanisms of detoxification of Cd involving soluble proteins. The presence of Cd detoxification mechanisms involving MTs was suspected when considering the chromatograms of metalloproteins from the digestive gland of the squids Nototodarus gouldi, T. pacificus and Ommastrephes bartrami from the Pacific Ocean (Tanaka et al 1983, Finger & Smith 1987, Castillo & Maita 1991). Later, these proteins were quantified in various cephalopod species from the Northern Atlantic Ocean (Bustamante et al. 2002b). In S.

officinalis from our study, cytosolic Cd was mainly associated with high molecular weight proteins and MTs seems to have a minor role in the binding of this metal (Figure 3). This result is in accordance with previous reported data for other cephalopod species. For example, most of the cytosolic Cd in the digestive gland of the squids *T. pacificus* and Onychoteuthis borealajaponica was bound to proteins weighing more than 70 kDa (Tanaka et al 1983, Castillo et al 1990). Only a small part of the soluble Cd was bound to low molecular weight proteins (<3 kDa) or to proteins of similar size to MT (10 kDa to 16 kDa). Moreover, Finger & Smith (1987) have also reported the occurrence of Cd-binding proteins with a high molecular weight (> to 70 kDa) in the digestive gland of the squid N. gouldi.

Similarly to Cd, Cu has been mainly found in the cytosol of the digestive gland cells of the squids N. gouldi (78 ± 10 %, Finger & Smith 1987) and T. pacificus (63 ± 5 %, Tanaka et al 1983) but poorly associated to hydrosoluble compounds in the squid Loligo forbesi (35 %, Craig & Overnell 2003) and in the octopus Octopus vulgaris (28 ± 17 %, Rocca 1969). The presence of high Cu concentrations in the digestive gland cells of S. officinalis has been revealed histochemically in specific structures called “spherules” (Martoja & Marcaillou 1993). These authors suggested that these metal-rich spherules would be made of complexed metallothionein-like proteins and would explain the high concentrations of Cu within the digestive gland. However, the presence of such structures has not been confirmed by other studies (Boucaud-Camou & Boucher-Rodoni 1983) and our results are not in accordance with such a hypothesis. Indeed, because of their size (i.e. several μm), such structures would be expected to sedimentate in the first or the second pellet fraction, containing in fact a low proportion of the total Cu (Table 2). In S. officinalis, Cu was mainly cytosolic, a main pool corresponding to high weight proteins which might contain haemocyanin molecules (Taylor and Anstiss 1999). However, Cu also appears to be bound to MT proteins and to a lower extent, to small size proteins and peptides (Figure 3).

To the best of our knowledge, no data on Co subcellular distribution in the digestive gland of cephalopod have been published to date. In S. officinalis, 64 % of this metal was associated to the cytosolic fraction, which is very similar to the results reported for the Bivalves Chlamys varia (76 %), Gafrarium tumidum (79 %), and Isognomon isognomon (65 %) in their digestive glands (Bustamante & Miramand 2005, Metian et al 2005). Low molecular weight proteins seem to have a major importance in binding Co (Figure 3). This result is consistent with those of Nakahara et al (1982) for O. vulgaris exposed to ^{60}Co via seawater, where the proteins involved in the binding of the radionuclide weighed less than 3kDa.

The predominant distribution of Ag in the insoluble fraction (*viz.* the non-cytosolic fractions) could be due to specific Ag storage/detoxification in the digestive gland. In various bivalves, Ag is trapped as non-toxic Ag_2S precipitates within their tissues (Ballan-Dufrançais et al 1985, Martoja et al 1989, Berthet et al 1990, 1992) and is mainly found associated to the organelle subcellular fraction (e.g. Bustamante & Miramand 2005). This mechanism of sequestration would therefore inhibit the potentially deleterious effects of the highly toxic Ag which is highly accumulated in the digestive gland of cephalopods (Martin & Flegel 1975, Miramand & Bentley, 1992). Specifically, the lysosomal and mitochondrial fraction appears to play a major role in the binding of Ag (Table 2). Our results concerning Ag are opposite to those of Tanaka et al (1983) for the squid T. pacificus for which $64 \pm 4\%$ of the metal was reported to be soluble and associated to small proteins (< 3kDa). The reasons of such a totally contrary result are difficult to identify as various biological and environmental factors could modify the subcellular distribution of a single metal within a group or a species, such as the phylogeny, the main pathway of incorporation (seawater *vs* food), the level of the metal accumulated, etc (e.g. Ueda et al 1985, Bustamante et al 2002b). This clearly points out the need to give light on the issue of Ag subcellular distribution using a wide range of cephalopod

species and controlled experimental conditions. Within the cytosolic fraction, Ag was mainly bound to high molecular weight proteins and to a lower extent to MTs.

Similarly to Ag, Pb has no biological functions. In the digestive gland of S. officinalis, most of this metal was found in the organelles (62%). In the digestive gland of the scallop *C. varia*, Pb was also mainly bound to organelles, i.e. 66% of the total metal burden (Bustamante & Miramand 2005). Similarly, in *Mytilus galloprovincialis* and *Modiolus modiolus*, Pb was mainly associated to the fraction containing nuclei, cellular debris and insoluble salts (Julshamm & Andersen 1983, Regoli & Orlando 1994). In these Bivalves, Pb is accumulated by endocytosis and precipitate as sulphur or phosphate salts inside the digestive cells (Coombs & George 1978) as well as in the extracellular compartments (Schulz-Baldes 1978). Lysosomes are deeply involved in the detoxification of Pb and lead to the formation of Pb salts representing the final stage of the lysosomal detoxification process (Simkiss 1977). In S. officinalis, no specific affinity among the different fractions has been shown (Table 2), suggesting that such a detoxification process is relatively limited, likely to be due to low Pb concentrations found in this species.

The subcellular distribution of Fe in S. officinalis with 96 % of the metal being bound to organelles, can only be compared to the 86% reported for T. pacificus (Tanaka et al 1983). However, the distribution among the organelle fraction is clearly different as in the cuttlefish 52% was bound to the nuclei fraction whereas in the squid, 42 % was bound to the microsome fraction. In Bivalves such as mussels or clams, Fe is also primarily associated with the nuclei fraction (Julshamm & Andersen 1983, Sullivan et al 1988, Regoli & Orlando 1994). In these molluscs, Mn has a similar subcellular distribution as Fe, which is actually not the case for cephalopods (Table 2). This difference in the distribution of Fe and Mn between the organelles could be due to the difference in the diet between carnivorous cephalopods and suspending or deposit feeder Bivalves. Indeed, Bivalves could ingest suspended/deposited

material enriched in Fe and Mn hydroxide particules (e.g. Bryan & Uysal 1978). Within the cytosolic fraction, Mn and Fe are the only elements not associated to MTs (Figure 3).

CONCLUSION

The subcellular distribution of heavy metals clearly varies depending on the considered element. For both essential and toxic metals, the sequestration in either the organelles or the cytosolic proteins could lead to specific accumulation. In this context, it is particularly striking that the different detoxification mechanisms for toxic Ag (mainly insoluble) and Cd (largely soluble) lead to their bioaccumulation at relatively high concentrations in the digestive gland of *S. officinalis*. In our conditions, a direct relationship between cytosolic metal and MT could only be established for Ag and Cu whereas Cd and Zn seems to mainly bind high (>70kDa) and low (< 4kDa) molecular weight proteins. Further studies should focus on the induction of MTs by the different metals inducing it synthesis in other invertebrates, i.e. Ag, Cd, Cu, Hg and Zn in controlled conditions in order to determine the dynamic of the detoxification processes in cephalopods.

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Table 1. Comparison of elemental concentrations ($\mu\text{g.g}^{-1}$ dwt) of Orchard-leaves standard, SRM 1571 (National Bureau of Standards) and fish flesh homogenate, MA-A-2 (International Agency of Atomic Energy) obtained in present study with certified values.

Standard	Ag	Cd	Co	Cu	Fe	Mn	Pb	Zn
Orchard leaves								
Present study	-	0.10 ± 0.05	0.17 ± 0.04	10 ± 1	272 ± 14	82 ± 7	38 ± 2	22 ± 6
Certified values	-	0.11 ± 0.02	(0.2)	12 ± 1	300 ± 20	91 ± 4	45 ± 3	25 ± 3
MA-A-2								
Present study	0.12 ± 0.01	0.069 ± 0.008	0.09 ± 0.04	3.4 ± 0.7	65 ± 5	0.62 ± 0.09	0.43 ± 0.14	35 ± 4
Certified values	0.10 ± 0.01	0.066 ± 0.004	0.08 ± 0.01	4.0 ± 0.1	54 ± 1	0.81 ± 0.04	0.58 ± 0.07	33 ± 1

(): recommended value

Table 2. Partition of the metals (%) among the different separated fractions from the digestive gland homogenates of the common cuttlefish *Sepia officinalis*.

Fractions	Ag	Cd	Co	Cu	Fe	Mn	Pb	Zn
Nuclei and brown bodies	19 ± 3	14 ± 8	11 ± 6	13 ± 9	52 ± 3	10 ± 4	20 ± 9	17 ± 8
Lysosomes and mitochondria	44 ± 3	11 ± 4	13 ± 1	12 ± 2	30 ± 5	31 ± 2	20 ± 3	20 ± 2
Microsomes	13 ± 4	23 ± 5	12 ± 3	19 ± 4	14 ± 2	32 ± 9	22 ± 4	23 ± 6
Total organelles	76 ± 10	48 ± 17	36 ± 10	44 ± 15	96 ± 10	73 ± 15	62 ± 16	60 ± 16
Cytosol	24 ± 2	52 ± 9	64 ± 9	56 ± 9	4 ± 1	27 ± 5	38 ± 8	40 ± 9

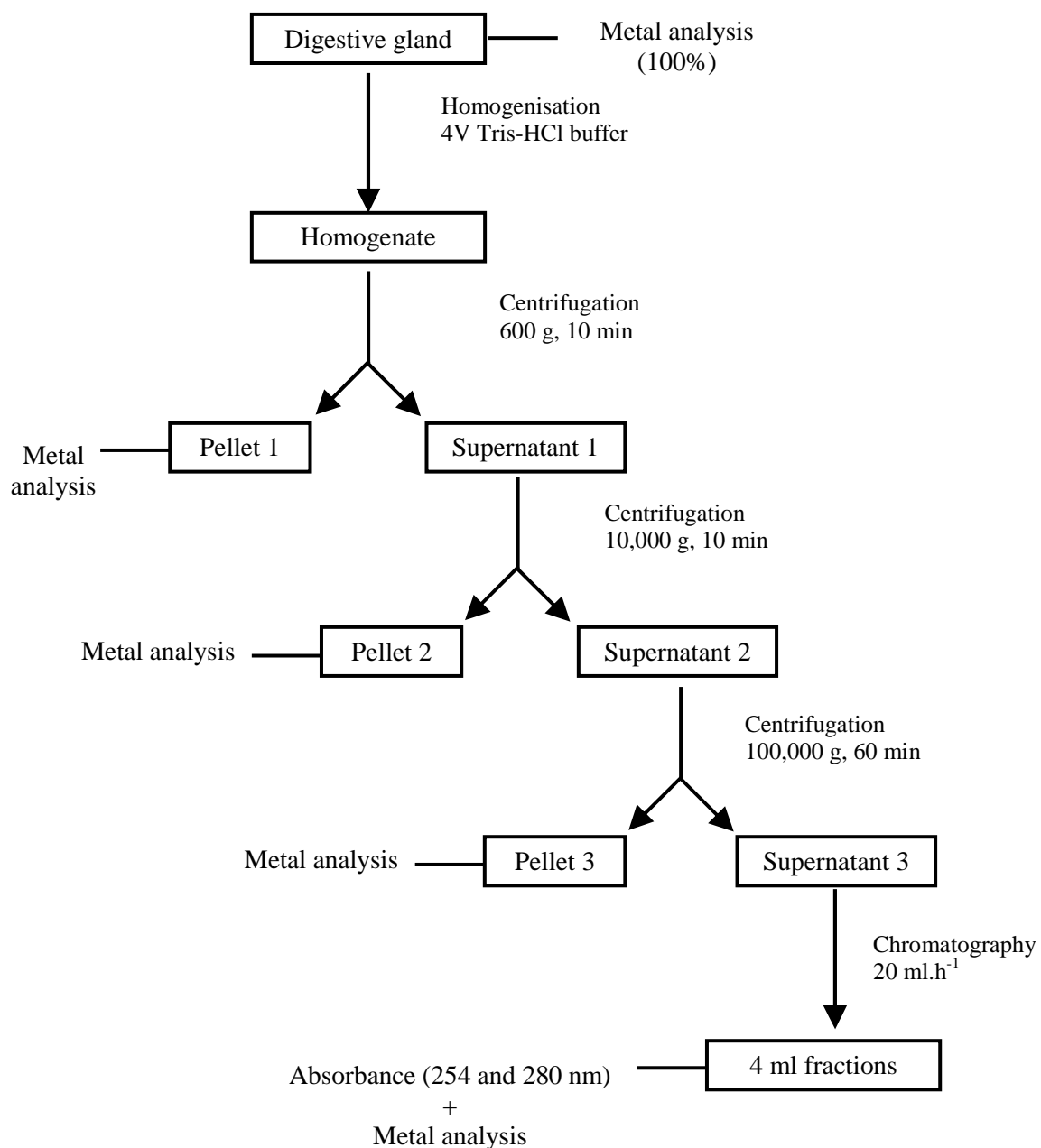


Figure 1. Procedure of the subcellular fractionation of the digestive gland of the cuttlefish *Sepia officinalis* from the Bay of Seine.

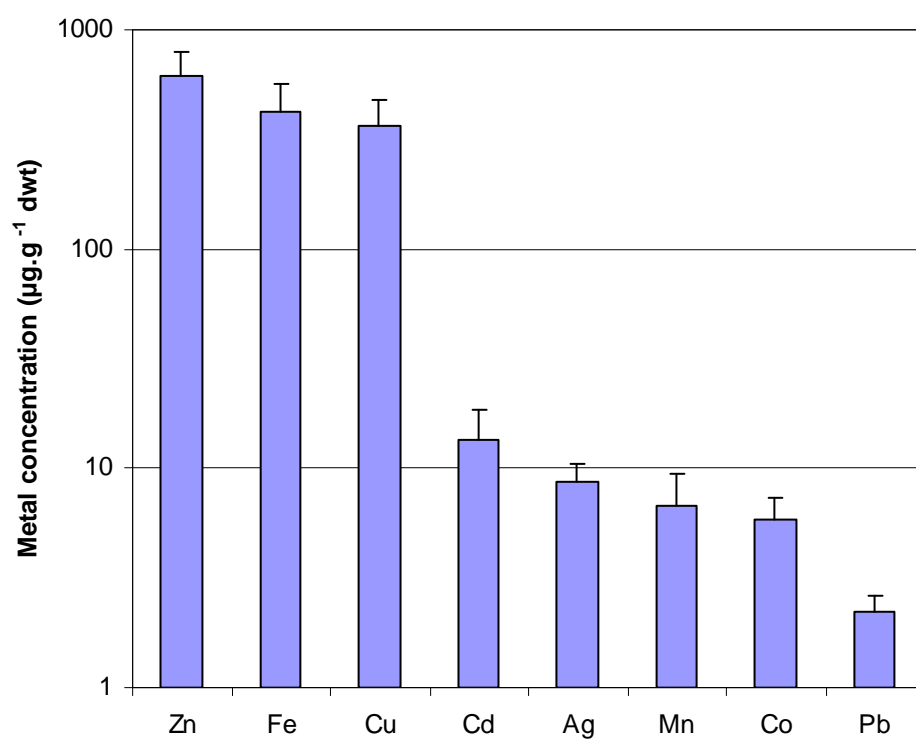


Figure 2. Levels of heavy metals ($\mu\text{g}\cdot\text{g}^{-1}$ dwt) in the digestive gland of the cuttlefish *Sepia officinalis* from the Bay of Seine.

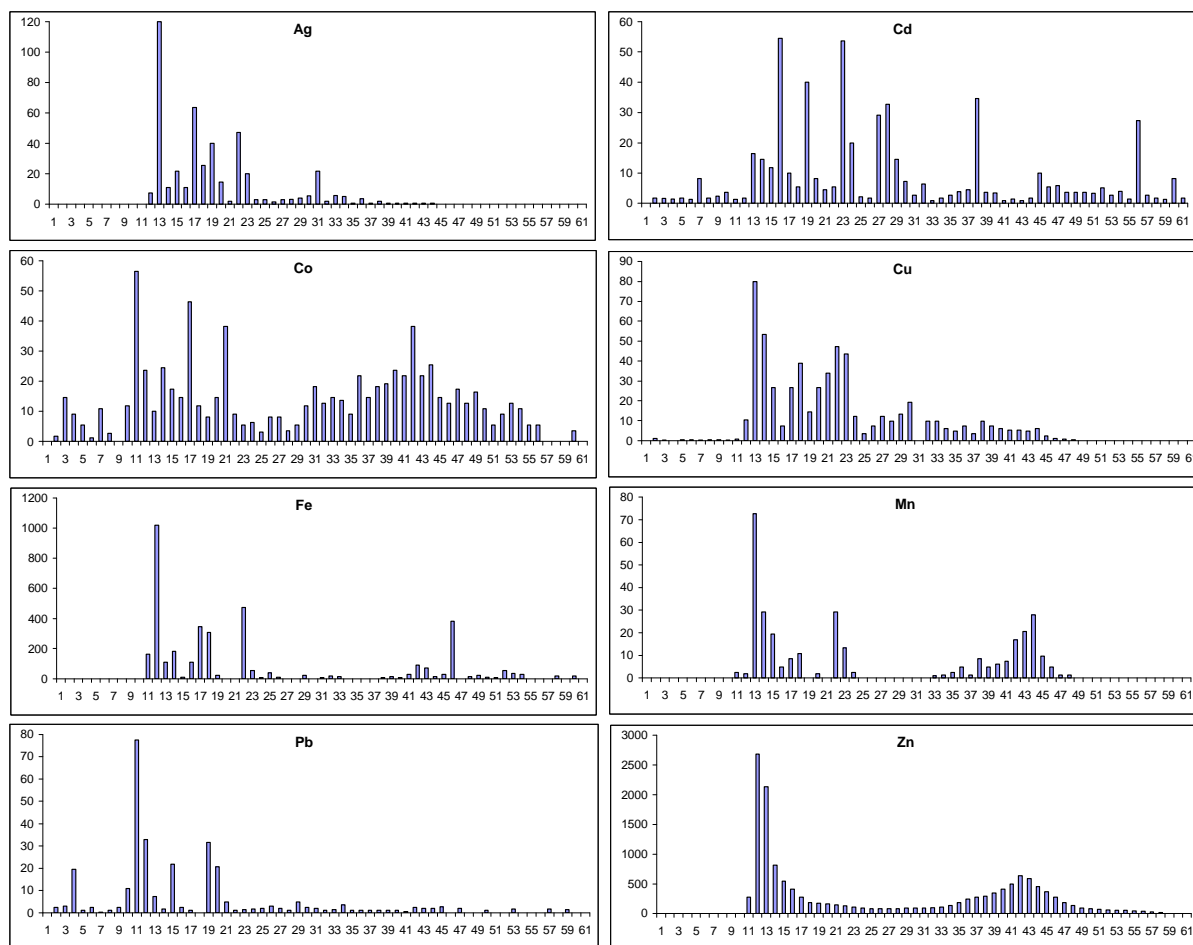


Figure 3. Metal profiles ($\mu\text{g.l}^{-1}$) after Sephadex G-75 gel chromatography of the cytosolic fractions of *Sepia officinalis* digestive gland.