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1 **Benthic foraminiferal ultrastructural alteration induced by heavy metals**

2
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15 16 **Abstract**

17 Heavy metals are known to cause deleterious effects on biota because of their toxicity,
18 persistence and bioaccumulation. Here, we briefly document the ultrastructural changes
19 observed in the miliolid foraminifer *Pseudotriloculina rotunda* (d'Orbigny in Schlumberger,
20 1893) and in the perforate calcareous species *Ammonia parkinsoniana* (d'Orbigny, 1839)
21 induced by exposure to one of three heavy metals (zinc, lead, or mercury). The exposure of
22 these two benthic foraminiferal species to the selected heavy metals appear to promote
23 cytological alterations and organelle degeneration. These alterations include a thickening of the
24 inner organic lining, an increase in number and size of lipid droplets, mitochondrial
25 degeneration, and degradation vacuoles and residual body proliferation. Some of these
26 alterations, including the thickening of the inner organic lining and the proliferation of lipids,
27 might represent defense mechanisms against heavy metal-induced stress.

28
29 **Key words:** protist, pollution, miliolid, ultrastructure, cytoplasm, *Ammonia*, *Pseudotriloculina*

30 31 **1. Introduction**

32 Benthic foraminifera are single-celled eukaryotes that are highly abundant in marine
33 environments. Traditionally, benthic foraminifera have been applied to paleoecological,
34 paleoenvironmental and paleoclimatological reconstructions and hydrocarbon exploration.

35 Their application has been also extended to environmental biomonitoring (for a review, see
36 Alve, 1995; Yanko et al., 1999) and they are now widely used as effective bioindicators in a
37 wide range of marine and transitional marine environments (e.g., Armynot du Châtelet and
38 Debenay, 2010; Frontalini and Coccioni, 2011; Schönfeld et al., 2012; Alve et al., 2016).

39 Although several ecological studies on benthic foraminifera have been performed over the
40 last 50-60 years, only a few have focused on the specific response to a single pollutant in terms
41 of tolerance, growth, reproduction and/or survival (i.e., Bresler and Yanko, 1995a,b; Morvan et
42 al., 2004; Saraswat et al., 2004; Le Cadre and Debenay, 2006; Nigam et al., 2009; Denoyelle et
43 al., 2012; van Dam et al., 2012a,b; Linshy et al., 2013; Nardelli et al., 2013; Frontalini et al.,
44 2015, 2016) and even more limited is the knowledge of ultrastructural changes induced by
45 exposure to pollutants (Table 1). Fewer ultrastructural studies on the effects of pollution on
46 foraminifera have so far been published as compared to those performed on other marine
47 organisms, ranging from other protists (Ismail et al., 2002; Debelius et al., 2009; Gomiero et
48 al., 2013; Miazek et al., 2015; Sures Kumar et al., 2015) to metazoans (e.g., Storelli and
49 Marcotrigiano 2000; Achard et al. 2004). In fact, most foraminiferal studies have focused more
50 on metals' incorporation into calcite for proxy calibration rather than on cellular responses or
51 alterations resulting from heavy metal exposure (i.e., de Nooijer et al., 2007; Munsel et al.,
52 2010; Nardelli et al., 2016; van Dijk et al., 2017a,b). Under these circumstances, further culture
53 and ultrastructural studies are required to better understand the specific biological response(s)
54 of foraminifera (Nigam et al., 2006), and the potential detoxification mechanisms against heavy
55 metals.

56 In benthic foraminifera, heavy metals have been suggested to promote 1) a thickening of the
57 inner organic lining (IOL); 2) an increase in the number and size of lipid droplets (LD); 3)
58 mitochondrial degeneration; 4) proliferation of degradation vacuoles (reported as
59 autophagosomes), lysosomes, and residual bodies in *Ammonia* species (Morvan et al., 2004; Le
60 Cadre and Debenay, 2006; Frontalini et al., 2015, 2016). Following LeKieffre et al. (this
61 volume), as the separation between degradation vacuoles containing external (food) or internal
62 (organelles) material is rather difficult on transmission electron microscopy (TEM) images, we
63 use the term degradation vacuoles. Alterations on LD (reported as lipidic vesicles) have been
64 attributed to a perturbation in the metabolic regulation of specimens exposed to copper (Cu)
65 (Le Cadre and Debenay, 2006). Similarly, a proliferation of LD, mainly neutral lipids (esterified
66 cholesterol and triglycerides), has been documented in specimens treated with Hg (Frontalini
67 et al., 2016). The IOL has been considered to protect the cell from xenobiotics (Leutenegger,

68 1977) and its thickening has reinforced the idea of a defense-like mechanism against pollutants
69 (Le Cadre and Debenay, 2006).

70 Among metals, zinc (Zn) is considered as an essential intracellular element and
71 micronutrient for eukaryotic life because it plays important roles in cellular proliferation,
72 metabolism, reproduction, enzymatic activity and protection against free radicals (e.g., Martín-
73 González et al., 2005; Gallego et al., 2007). However, at high concentrations Zn becomes toxic
74 and produces cellular damage (e.g., Gallego et al., 2007), mainly due to oxidative stress (e.g.,
75 de Freitas Prazeres et al., 2011). Lead (Pb) has been regarded among the most damaging
76 elements to organisms as it mimics other biologically essential metals by substituting for Ca,
77 Mg, Fe, Zn, and Na (Lidsky and Schneider, 2003; Flora et al., 2012). Because of its non-
78 biodegradable nature, Pb is known for its prolonged persistence in the environment (Flora et
79 al., 2012). Lead, like Zn, also promotes the generation of reactive oxygen species (ROS) that
80 might result in damage to biomolecules (Flora et al., 2012). Mercury (Hg) and its compounds
81 are extremely toxic and has been considered as one of the most harmful metals for biota
82 (Clarkson and Magos, 2006; Eisler, 2006). Mercury is also reported to cause oxidative stress
83 (McElwee et al., 2013).

84 The main aim of this contribution for this Benthic Foraminiferal Ultrastructure Atlas special
85 issue is to present the most commonly noted ultrastructural changes observed in two species of
86 benthic foraminifera, *Ammonia parkinsoniana* and *Pseudotriloculina rotunda*, after acute
87 exposure to these two non-essential (Hg and Pb) and one essential (Zn) heavy metals.

88

89 **2. Materials and methods**

90 **2.1 Experimental conditions**

91 Three experiments were conducted with the selected metals (Zn, Pb and Hg).

92 In unpolluted seawater Zn concentrations are generally < 10 µg/L (Eisler, 1993) but in highly
93 polluted areas concentrations up to 5 mg/L have been reported (Reddy et al., 2005). To test the
94 effect of Zn, a culture experiment exposed the miliolid species *Pseudotriloculina rotunda*
95 (d'Orbigny in Schlumberger, 1893) to 50 mg/L of Zn for 24h. This concentration was chosen
96 on the basis of the results obtained by Nardelli et al. (2013) on the same species. Because a
97 concentration of 100 mg Zn/L was lethal to this species after one week of treatment and <50%
98 of specimens died during a 7-week incubation at 10 mg Zn/L, an intermediate concentration of
99 50 mg Zn/L was chosen for the acute exposure. A preliminary test was also performed to ensure
100 that all specimens survived 24h of exposure to this concentration of Zn. Specimens were
101 isolated from culture batches (see Nardelli et al., 2013 for more details) and maintained at 18°C;

102 only those with active reticulopods were used as inoculates and checked for pseudopodial
103 activity before starting the experiment. Zinc solution was prepared, just before starting the
104 experiment, with ultrapure salts ($ZnSO_4 \cdot 7H_2O$, from Sigma) diluted in natural seawater from
105 an established control site (Portonovo, Ancona, Italy) previously filtered at $0.42 \mu m$ and stored
106 in the dark, at $4^\circ C$. Salinity (37) and pH (8.02) were previously measured. Specimens were
107 picked from culture batches, that were normally kept at $15^\circ C$ and gradually raised to $18^\circ C$ in
108 the week preceding the experiment. Fifteen specimens of *P. rotunda* were randomly picked
109 from the pool to be exposed to 50 mg/L and twelve were maintained in control conditions
110 (seawater). Untreated (i.e., control) and treated (i.e., Zn) foraminiferal specimens were
111 incubated at $18^\circ C$, for 24h and then separately processed for TEM analyses.

112 The Pb experiment was based on *Ammonia parkinsoniana* (d'Orbigny, 1839) and has been
113 fully described in Frontalini et al. (2015). In brief, specimens were cultured in sediments
114 exposed to one of three concentrations of Pb (1 ppb, 1 ppm, or 10 ppm) or the control (no lead),
115 up to eight weeks. Similarly, the Hg-based experiment was performed on *A. parkinsoniana*,
116 exposed to one of three concentrations of Hg (1 ppb, 1 ppm, or 100 ppm) or the control up to
117 12 weeks (Frontalini et al., 2016).

118

119 **2.2 Sample preparation for TEM analyses**

120 Specimens of *P. rotunda* were prepared for TEM observation following the protocol
121 described by Martín-González et al. (2005), modified after Le Cadre and Debenay (2006).
122 Briefly, after incubation, foraminiferal specimens were pre-fixed in a solution of glutaraldehyde
123 2.5% (v/v) (Sigma) in sodium cacodylate buffer (100 mM, pH 7.2, TAAB Laboratories
124 Equipment Ltd), for 1 hour. Then specimens were exposed to 0.1M ethylenediaminetetraacetic
125 acid (EDTA) for 36h to remove the calcareous test (following Le Cadre and Debenay 2006).
126 The cells were then rinsed 3 times in 100 mM sodium cacodylate buffer and post-fixed in a
127 0.5% solution of OsO_4 (v/v; TAAB) in sodium cacodylate buffer (100 mM, pH 7.2) for 45
128 minutes on ice. Fixed cells were then contrasted in an aqueous solution of 1% (v/v) uranyl
129 acetate (TAAB) for 1 hour, dehydrated in a graded series of acetone baths (25%, 50%, 75%,
130 and 3 times 100% (v/v) in Millipore water), for no less than 20-30 minutes for each step.
131 Foraminifera were then embedded in Embed Low Viscosity Resin, following manufacturer's
132 instructions. These various manipulations were performed in small microcentrifuge tubes
133 (Eppendorf type) using micropipettes. Samples were then processed at the National Center for
134 Microscopy (Complutense University of Madrid). Initially, semi-thin sections ($1 \mu m$) of the
135 specimens were stained with 1% (v/v) toluidine blue to check for cell integrity. Ultra-thin (50

136 nm) sections were collected on 200-mesh copper grids and contrasted with aqueous 8% uranyl
137 acetate (in ethanol 30%) and 0.7% lead citrate solutions, and then examined with a JEM 1010
138 JEOL Electron Microscope, at 75kV.

139 *Ammonia parkinsoniana* preparation for TEM analyses is described in Frontalini et al. (2015,
140 2016). Briefly, specimens were fixed with 2.5% glutaraldehyde (TAAB, England, UK) in
141 Artificial Sea Water (ASW) for 3 h at 4°C, and decalcified with 0.1 M EDTA for 36 h. After
142 5 washings with ASW, specimens were post-fixated with 1% osmium tetroxide (OsO₄; EMS,
143 Hatfield, PA) in ASW for 2 h at room temperature. Specimens were then dehydrated in a graded
144 series of ethanol baths, from 50% to 100%, immersed twice in propylene oxide (10 minutes
145 each; EMS, Hatfield, PA) and embedded in epoxy resin (Durcupan Araldite, SIGMA, UK).
146 Foraminifera were ultimately sectioned using an ultramicrotome (LKB, 2088 Ultratome®V).
147 Thick sections of 1 µm were stained with 1% toluidine blue in distilled water at 60°C to provide
148 an overview at the light-microscope level. Thin sections (100 nm), collected on 300-mesh
149 nickel grids, were stained with 3% aqueous uranyl acetate and Reynold's lead citrate solutions
150 and finally observed with a Philips CM10 electron microscope at 80 kV.

151

152 **3. Results**

153 The comparison between control (Fig. 1A) and Zn-treated (Fig. 1B-F) specimens of *P.*
154 *rotunda* revealed important ultrastructural alterations in zinc-treated individuals but not in
155 control specimens. These alterations included the presence of large numbers of residual bodies,
156 which contained irregular concentric or juxtaposed masses of membranes that can form into a
157 vacuole. Moreover, what were interpreted to be numerous cytoplasmic degradation vacuoles
158 and electron-dense granules were visible in Zn-treated cells (Fig. 1B, C and D). Golgi apparatus
159 (Fig. 1C-D) and mitochondria (Fig. 1B, D and E) were degraded in treated specimens compared
160 to those of the control specimens, where intact organelles (mitochondria and peroxisomes) were
161 commonly observed (Fig. 1A). Some clay particles as interpreted by Goldstein and Corliss
162 (1994) or mineral flake-like crystals were visible both in treated (Fig. 1F) and control specimens
163 and have been interpreted as mica flakes that entered the cell by endocytosis, possibly before
164 the isolation of specimens from natural sediment for culture.

165 Control specimens of *A. parkinsoniana* appeared as expected in “normal” foraminiferal cells
166 (see, e.g., LeKieffre et al. this volume), having intact vacuole membranes, mitochondria,
167 peroxisomes, and residual bodies (Fig. 2A). On the contrary, several ultrastructural alterations
168 were observed in Pb-treated specimens, particularly those exposed to the highest
169 concentrations, including cytoplasmic degradation (Fig. 2B). In fact, the membranes delimiting

170 organelles were ruptured, causing the cytosol to appear to fuse between structure. At higher
171 magnification, mitochondria did not show the typical peripheral double membrane integrity,
172 and they appeared swollen with poorly preserved cristae (Fig. 2D) when compared to control
173 specimens (Fig. 2C). Lipid droplets of Pb-treated specimens appeared to have a more irregular
174 outline and to be more electron-dense (Fig. 2F) than LD of control specimens (Fig. 2E).
175 Similarly, Hg-treated specimens showed numerous morphological alterations compared to
176 control specimens. These alterations included cytoplasmic degradation (Fig. 3A), a more
177 electron-dense core in lipids (Fig. 3B), degraded mitochondria (Fig. 3C), and a number of
178 structures interpreted as vacuoles (Fig. 3A).

179

180 **Discussion**

181 Our results suggest that the ultrastructural alterations induced by exposure to different heavy
182 metals on different benthic foraminiferal species are quite similar, regardless of the involved
183 metal, at least for the three tested divalent ions and the two foraminiferal species. The three
184 tested metals are known to induce the production of reactive oxygen species (ROS) that
185 represent a serious threat to cell fitness during exposure. The ROS are mainly produced in
186 mitochondria (see review by Murphy 2009) and when an excess is produced, mitochondria
187 become dysfunctional or undergo necrosis (which leads to cell- “aging”). The degradation of
188 mitochondria observed in our treated samples is very similar to the ones already reported for
189 several species of ciliates (i.e., Pyne et al., 1983; Martín-González et al., 2005) and could
190 represent an advanced phase of necrosis of these organelles after exposure. This hypothesis also
191 agrees with the results of de Freitas Prazeres et al. (2011) who quantified, for the first time,
192 several biomarkers of oxidative stress (antioxidant capacity, lipid peroxidation,
193 metallothionein-like proteins concentration and total superoxide dismutase activity) in a
194 symbiont-bearing foraminifer, *Amphistegina lessonii*, exposed to Zn. However, it is noted that
195 some foraminiferal species, which thrive within the chemocline of marine sediments where
196 ROS are produced, possess peroxisomes complexed with the endoplasmic reticulum and can
197 cope with ROS (Bernhard and Bowser, 2008).

198 The presence of higher number of degradation vacuoles (probably autophagosomes) and
199 residual bodies in the cytoplasm of metal-treated specimens could therefore be the consequence
200 of enhanced stressful conditions and represent the tendency of the organism to degrade
201 organelles like mitochondria altered by ROS production. Increased number of degradation
202 vacuoles has been previously reported in other eukaryotic organisms, where they are generally
203 interpreted as cellular stress signals (e.g., Krawczynska et al. 1989; Martín-González et al.

204 2005). Traditionally, the formation of autophagosomes is considered a specific mechanism
205 induced by starvation or nutritional stress (Klionsky and Ohsumi 1999), for example in yeasts
206 (Abeliovich and Klionsky 2001) and ciliates (Gutiérrez et al. 2001; Gutiérrez and Martín-
207 González 2002). However, it is also known that autophagy plays a major role in the degradation
208 of altered organelles. An increased number of residual bodies has been reported by Le Cadre
209 and Debenay (2006) in two different species of *Ammonia* exposed to Cu. Those authors
210 hypothesized a role of the residual bodies in detoxifying the metals by storing and neutralizing
211 them. Dedicated studies to map metal distributions in foraminiferal cytoplasm are warranted.

212 A thickening of inner organic lining (IOL) or cell membrane was observed in *Ammonia*
213 *parkinsoniana* exposed to both Hg and Pb (Frontalini et al., 2015). This result is far from rare.
214 In fact, several authors previously reported similar observations in different foraminiferal
215 species, exposed to different metals. For example, Le Cadre and Debenay (2006) who exposed
216 *Ammonia beccarii* (Linnaeus, 1758) and *Ammonia tepida* (Cushman, 1926) to copper (up to
217 500 µg/L), in culture conditions, reported a thickening of the IOL with a fibrous and stratified
218 appearance particularly at the basal part of the pores. Similarly, a thickening of the IOL with an
219 increase of fibrous material and the proliferation of residual bodies is documented in
220 morphologically deformed *A. tepida* specimens after exposure to oil by Morvan et al. (2004).
221 The IOL, which consists of a complex polysaccharide and glycoproteins bound together in a
222 complex macromolecular structure, represents the matrix between the test and the cytoplasm
223 (Splinder, 1978; Ní Fhlaithearta et al., 2013) that is supposed to have an important role in
224 foraminiferal biomineralization (Langer, 1992; Erez, 2003, Sabbatini et al., 2014). Morvan et
225 al. (2004) suggested that this structure could also have a major role in protecting the cytoplasm,
226 and that a thickening of the IOL may therefore represent a defense mechanism adopted by
227 foraminifera to protect the cell against potential toxicants like heavy metals (i.e., Cu and Pb),
228 and also organic compounds (Sen Gupta et al., 1997; Morvan et al., 2004; Le Cadre and
229 Debenay, 2006; Frontalini et al., 2015). Similar modification of the IOL was also noted by Sen
230 Gupta et al. (1997) for *Cassidulina carinata* Silvestri, 1896 collected in *Beggiatoa* mats around
231 bathyal hydrocarbon seeps in the Gulf of Mexico as well as by Koho et al. (this volume) for
232 *Ammonia* spp. in response to anoxic conditions.

233 A proliferation of abnormally large LD (reported as lipidic vesicles) and an increase in the
234 number of residual bodies in response to copper exposure were noted by Le Cadre and Debenay
235 (2006). The increase of number and size of LD was suggested to be a perturbation in the
236 regulation metabolism of foraminiferal specimens contaminated by copper (Le Cadre and
237 Debenay, 2006) as also reported in other organisms (Prevot and Soyer-Gobillard, 1986). A

238 proliferation of neutral lipids in the form of LD was documented in specimens of *A.*
239 *parkinsoniana* exposed to Hg (Frontalini et al., 2016) as well as in other organisms (microalgae,
240 lichens, rat, grey mullet, silver catfish hepatocytes) when exposed to contaminants. Lipid
241 droplets are also hypothesized to sequester toxicants in order to protect cells (Murphy et al.,
242 2008; Rowan-Carroll et al., 2013).

243

244 **4. Conclusion**

245 Mercury, lead and zinc are important metallic pollutants that, to different degrees, are
246 considered potentially harmful elements for biota. On the basis of our results and literature data,
247 we here present a synopsis of the ultrastructural changes of two benthic foraminiferal species,
248 *A. parkinsoniana* and *P. rotunda* exposed to Hg and Pb, and Zn, respectively. The exposure of
249 the specimens to these heavy metals seems to promote cytological alterations and organelle
250 degeneration. These alterations include a thickening of the inner organic lining, an increase in
251 the number and size of lipid droplets, mitochondrial degeneration, as well as degradation
252 vacuole and residual body proliferations. These alterations suggest cytological oxidative stress
253 induced by the exposure to the tested metals, and some alterations in particular, i.e., thickening
254 of IOL, degradation vacuole, and lipids, are interpreted as potential defense mechanisms against
255 heavy metal-induced stress.

256

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266

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453

454 **Table and Figures caption**

455 **Table 1.** Compilation of experimental results in previous literature about the effects on
456 foraminifera, after exposure to certain pollutants (pollutant, concentration, duration, species,
457 Transmission Electron Microscopy (TEM) and reference).

458

459 **Figure 1.** TEM micrographs of *Pseudotriloculina rotunda*. Low magnification view (A) of a
460 control specimen incubated in natural seawater. Higher magnification views of Zn-treated
461 specimens (B-F). Mitochondria (m), degraded mitochondria (m*), lipid droplet (li), Golgi
462 apparatus (g), degradation vacuole (dv), peroxisome (p), electron-dense granules (e) and clay
463 platelets (c). Scale bars: A-D: 1 μm ; E-F: 100 nm.

464

465 **Figure 2.** TEM micrographs of *Ammonia parkinsoniana*. Low magnification views of
466 foraminiferal cytoplasm of control (A) and Pb-treated (B) specimens. Lipid droplet (li), vacuole
467 (v) and residual body (rb). Higher magnification views of intact (C, control) and degraded (D,

468 Pb-treated) mitochondria, lipid droplets in untreated (E) and Pb-treated (F) specimens.
469 Peroxisome (inset in B). Scale bars: A, B: 1 μm (inset 125 nm); C, D: 50 nm; E, F: 200 nm.

470

471 **Figure 3.** TEM micrographs of *Ammonia parkinsoniana*. Low magnification view of
472 foraminiferal cytoplasm of Hg-treated (A) specimens. Higher magnification views of lipid
473 droplets (B) with electron-dense cores and degraded mitochondria (C). Lipid droplet (li),
474 vacuole (v) and mitochondria (m). Scale bars: A: 1 μm ; B: 200 nm; C: 100 nm.

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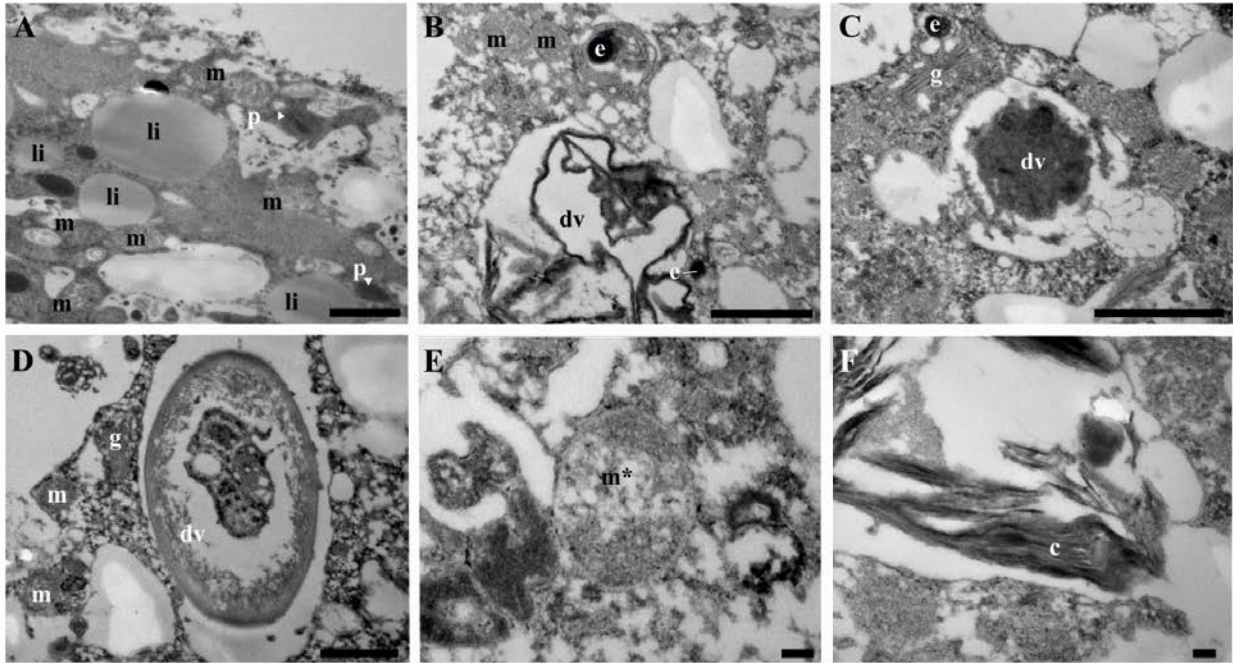
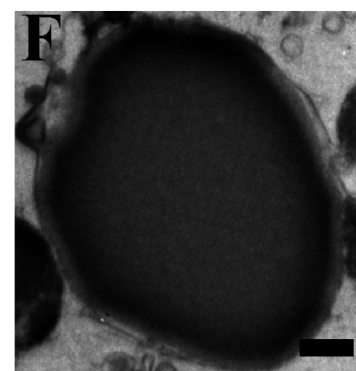
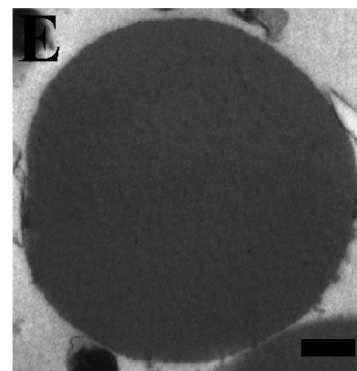
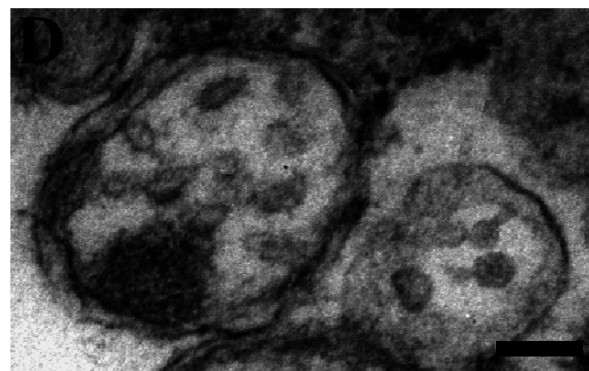
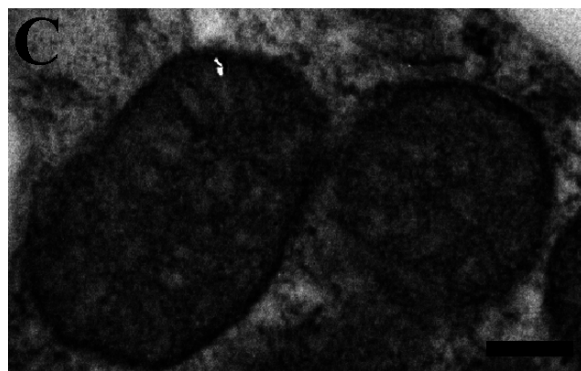
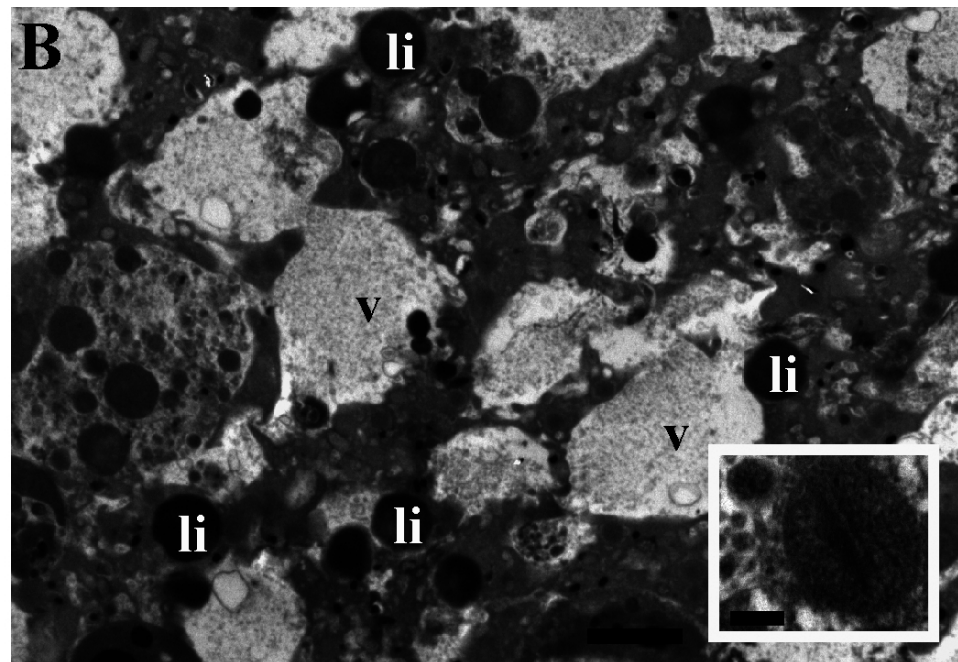
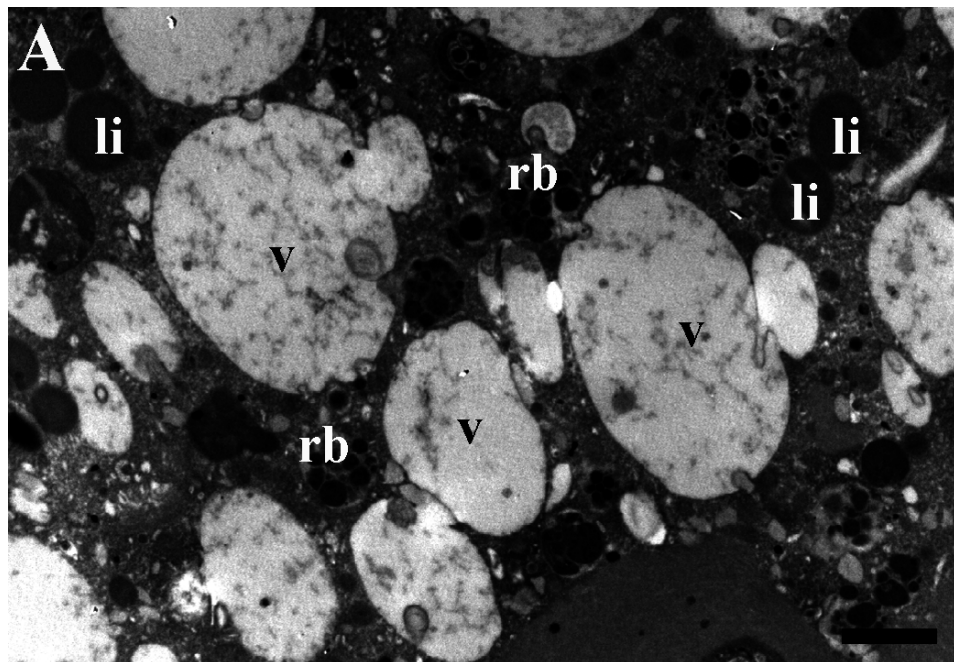


Figure 1.



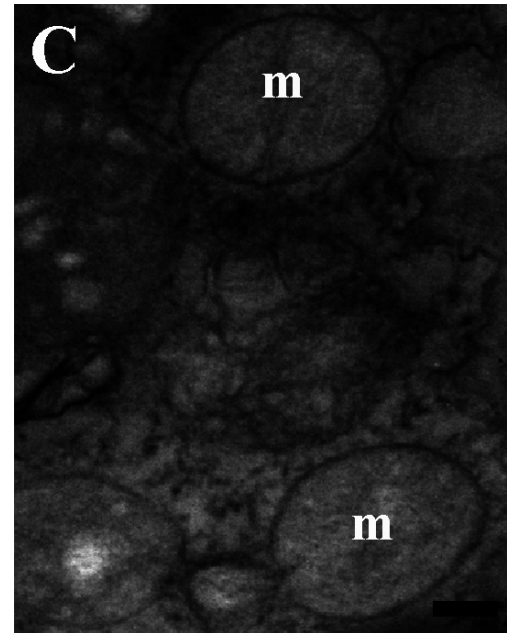
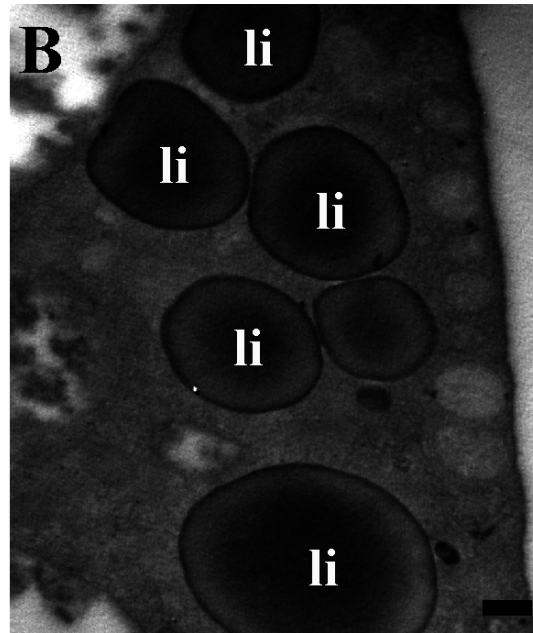
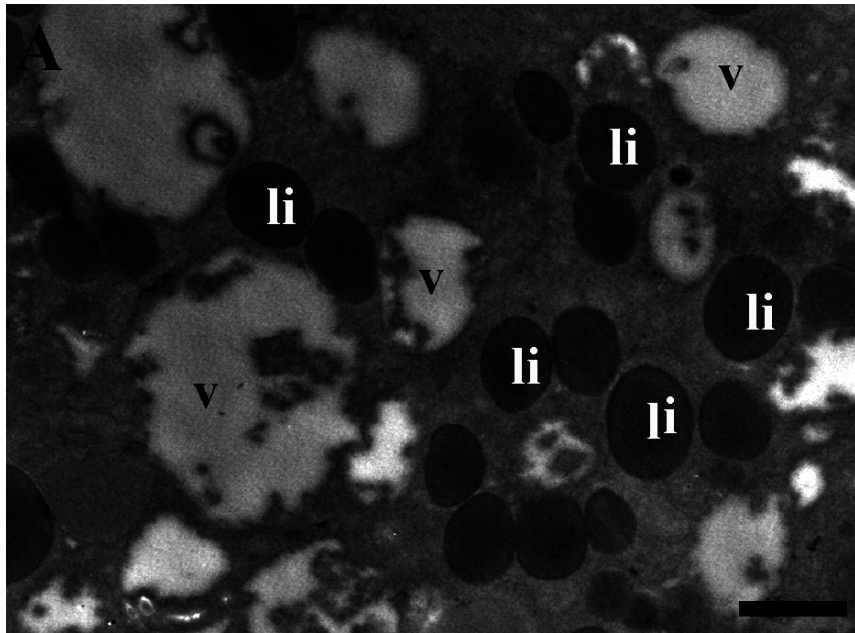


Table 1.

Pollutant	Concentration	Duration	Species	TEM	Reference
Cd, Cu and Hg	up 1000 μM	24 hours	<i>Pararotalia spinigera</i>	No	Bresler and Yanko (1995a)
Mixed compounds	up 1000 μM	up to 4 hours	<i>Pararotalia spinigera</i> and <i>Rosalina macropora</i>	No	Bresler and Yanko (1995b)
Oil	up to 72 mg/100 mL	up to 12 months	<i>Ammonia tepida</i>	Yes	Morvan et al. (2004)
Hg	up to 260 ng/L	100 days	<i>Rosalina leei</i>	No	Saraswat et al. (2004)
Cu	up to 500 $\mu\text{g/L}$	up to 12 months	<i>Ammonia beccarii</i> and <i>Ammonia tepida</i>	Yes	Le Cadre and Debenay (2006)
Cu	up to 20 $\mu\text{mol/L}$	up to 2 months	<i>Ammonia tepida</i> and <i>Heterostegina depressa</i>	No	de Nooijer et al. (2007)
Hg	up to 300 ng/L	ca. 40 days	<i>Rosalina leei</i>	No	Nigam et al. (2009)
Ni, Cu, and Mn	up to 3290 nmol/L	82 days	<i>Ammonia tepida</i>	No	Munsel et al. (2010)
Zn	up to 93.4 $\mu\text{g/L}$	48 hours	<i>Amphistegina lessonii</i>	No	de Freitas Prazeres et al. (2011)
Cd	up to 200 mg/L	up to 30 days	<i>Ammonia tepida</i>	No	Denoyelle et al. (2012)
Oil	up to 5 g/L	up to 30 days	<i>Ammonia tepida</i>	No	Denoyelle et al. (2012)
Drilling muds	up to 100 mg/L	up to 30 days	<i>Ammonia tepida</i>	No	Denoyelle et al. (2012)
Diuron (symbiont response)	up to 100 μL	up to 96 hours	<i>Heterostegina depressa</i> , <i>Amphistegina radiata</i> , <i>Alveolinella quoyi</i> , <i>Calcarina mayorii</i> , <i>Operculina ammonoides</i> , <i>Heterostegina depressa</i> , <i>Marginopora vertebralis</i> , <i>Marginopora vertebralis</i> , <i>Sorites orbiculus</i> , <i>Peneropolis planatus</i> , <i>Peneropolis antillarum</i> , <i>Parasorites marginalis</i> and <i>Elphidium sp.</i>	No	Van Dam et al. (2012a)
Diuron (symbiont response)	up to 3 $\mu\text{g/L}$	up to 96 hours	<i>Heterostegina depressa</i> , <i>Calcarina mayorii</i> , <i>Alveolinella quoyi</i> , <i>Marginopora vertebralis</i> and <i>Peneroplis planatus</i>	No	Van Dam et al. (2012b)
Cd	up to 14 $\mu\text{g/L}$	up to 21 days	<i>Pararotalia nipponica</i>	No	Linshy et al. (2013)
Zn	up to 100 mg/L	up to 10 weeks	<i>Pseudotriloculina rotunda</i>	No	Nardelli et al. (2013)

Pb	up to 10 mg/L	up to 2 months	<i>Ammonia parkinsoniana</i>	Yes	Frontalini et al. (2015)
Hg	up to 100 mg/L	up to 3 months	<i>Ammonia parkinsoniana</i>	Yes	present paper
Zn	50 mg/L	24 hours	<i>Pseudotriloculina rotunda</i>	Yes	present paper
Hg	up to 100 mg/L	up to 3 months	<i>Ammonia parkinsoniana</i>	Yes	present paper