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

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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 vacuole (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°C. Salinity (37) and pH (8.02) were previously measured. Specimens were  
107 picked from culture batches, that were normally kept at 15°C and gradually raised to 18°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°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 (OsO<sub>4</sub>; 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<sup>®</sup>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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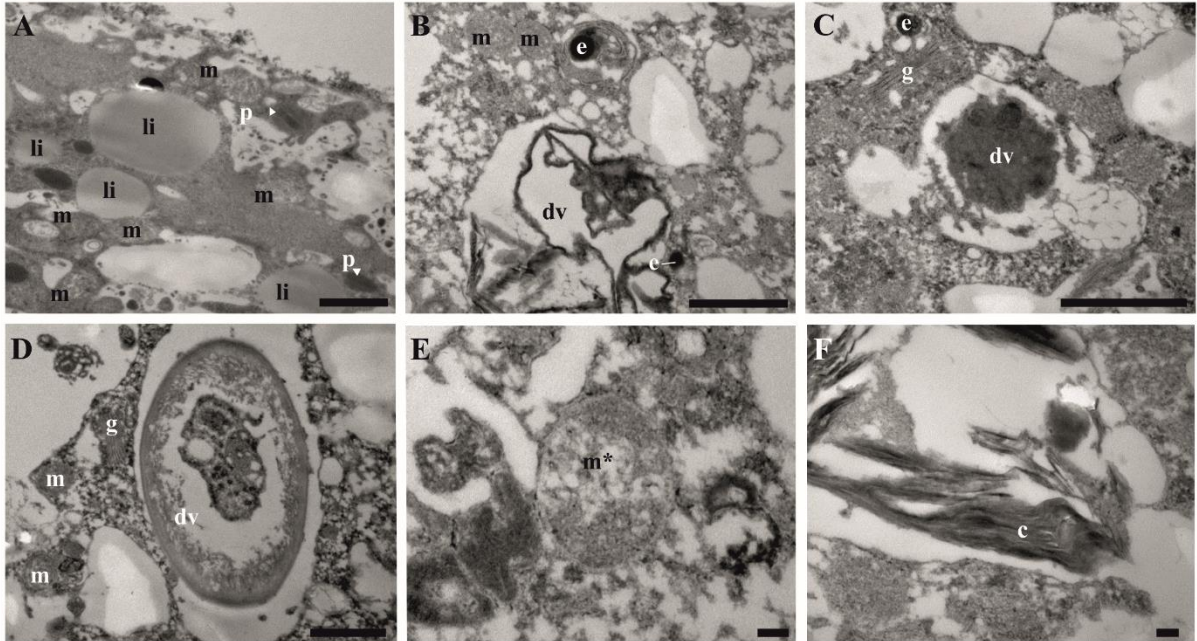
### 453 Table and Figures caption

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

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 µg/l	up to 12 months	<i>Ammonia beccarii</i> and <i>Ammonia tepida</i>	Yes	Le Cadre and Debenay (2006)
Hg	up to 300 ng/l	ca. 40 days	<i>Rosalina leei</i>	No	Nigam et al. (2009)
Zn	up to 93.4 µg/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/l	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</i> sp.	No	Van Dam et al. (2012a)
Diuron (symbiont response)	up to 3 µg/l	up to 96 hours	<i>Heterostegina depressa</i> , <i>Calcarina mayorii</i> , <i>Alveolinella quoyi</i> , <i>Marginopora vertebralis</i> and <i>Peneropolis planatus</i>	No	Van Dam et al. (2012b)
Cd	up to 14 µg/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

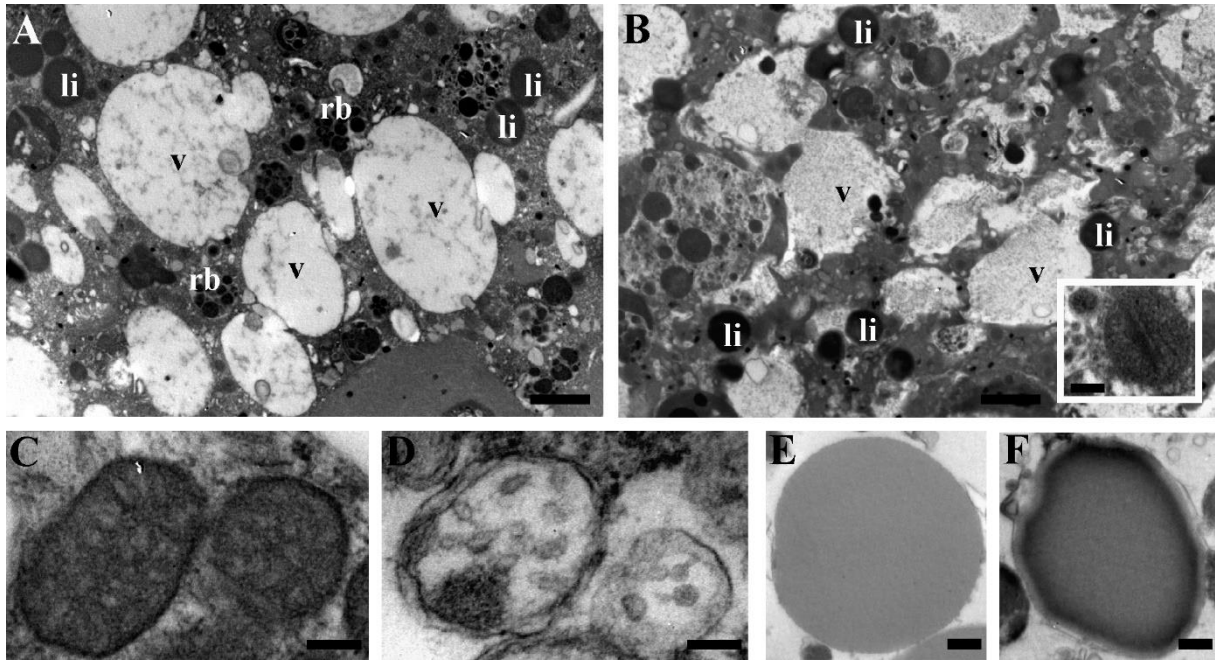
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458 **Figure 1.** TEM micrographs of *Pseudotriloculina rotunda*. Low magnification view (A) of a  
 459 control specimen incubated in natural seawater. Higher magnification views of Zn-treated  
 460 specimens (B-F). Mitochondria (m), degraded mitochondria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



463  
 464 **Figure 2.** TEM micrographs of *Ammonia parkinsoniana*. Low magnification views of  
 465 foraminiferal cytoplasm of control (A) and Pb-treated (B) specimens. Lipid droplet (li), vacuole  
 466 (v) and residual body (rb). Higher magnification views of intact (C, control) and degraded (D,  
 467 Pb-treated) mitochondria, lipid droplets in untreated (E) and Pb-treated (F) specimens.  
 468 Peroxisome (inset in B). Scale bars: A, B: 1  $\mu$ m (inset 125 nm); C, D: 50 nm; E, F: 200 nm.



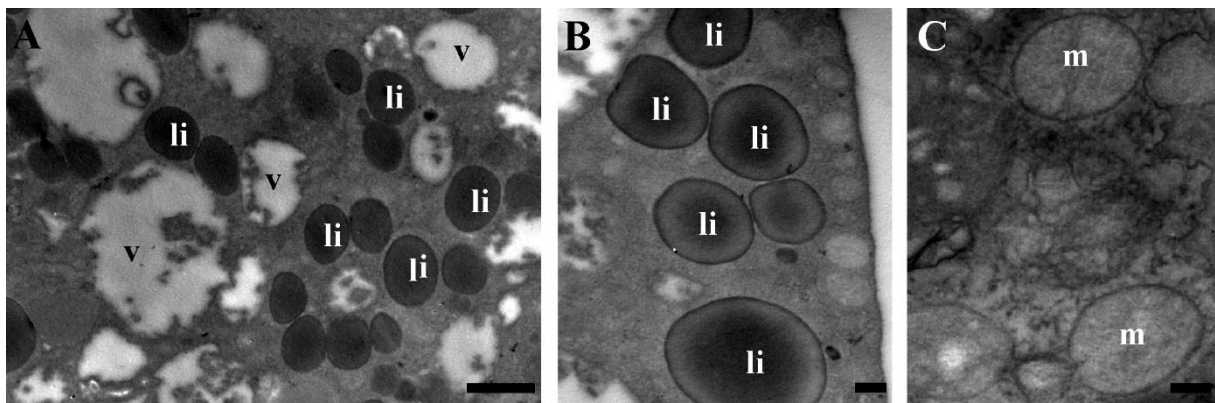


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471 **Figure 3.** TEM micrographs of *Ammonia parkinsoniana*. Low magnification view of  
 472 foraminiferal cytoplasm of Hg-treated (A) specimens. Higher magnification views of lipid  
 473 droplets (B) with electron-dense cores and degraded mitochondria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

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