The use of biomimetic tools for water quality monitoring: passive samplers versus sentinel organisms

M. Morrone · **N. E. Cappelletti** · **L. M. Tatone** ·
M. J. Astoviza · **J. C. Colombo**

Abstract To evaluate the performance of artificial and biological integrative sampling strategies, dissolved concentrations of polychlorinated biphenyl (PCBs) and metals (Cu and Mn) were measured with passive sampler devices (PSD) enclosing hydrophobic and chelating phases, and Asiatic clams were transplanted to a polluted area in the Rio de la Plata. Water concentrations based on PSD were compared with spot water samples collected at 0, 15, 32, and 63 days of exposition. PCBs and metals displayed linear accumulation kinetics both in PSDs and bivalves, but with different slopes. PCBs slopes were 2–27 times higher in bivalves (0.3–7.9 vs. 0.1–2.6 ng g^{-1} d^{-1} in PSDs) reflecting active filtration and uptake from particles, colloids, and dissolved phase, whereas metal slopes were 2.5–11 times lower in bivalves (0.04–0.18 vs. 0.10–2.00 µg g^{-1} d^{-1} in PSDs) suggesting metal bioregulation. Truly dissolved PCB concentrations from PSD (4.0–6.9 ng.l^{-1}) represented average 13±4 % of PCB concentrations from filtered spot water samples reflecting the operational discrimination of the PSD diffusion membrane on competing dissolved and colloidal organic phases. In contrast, PSD dissolved Cu concentrations (2.3–8.2 µg l^{-1}) were equivalent to the spot water values (3.2–3.8 µg l^{-1}) suggesting that Cu speciation was adequately integrated by passive sampling. On the other hand, PSD-dissolved Mn concentrations (49±38 µg l^{-1}) were higher ($p<0.05$) than those from spot water samples (7±11 µg l^{-1}) possibly due to phase changes of Mn distribution related to variable redox conditions in the water. Results evidenced that the PSDs are efficient accumulators of metals and organic compounds from the truly dissolved fraction.

M. Morrone (✉) · L. M. Tatone · M. J. Astoviza ·
J. C. Colombo
Laboratorio de Química Ambiental y Biogeoquímica, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Avenida Calchaquí km 23 500, (C1888) Florencio Varela, Buenos Aires, Argentina
e-mail: manuelmorrone@gmail.com

M. Morrone
Ministerio de Ambiente y Desarrollo Sostenible de la Nación, San Martin 451, (C1004) Autonomous City of Buenos Aires, Argentina

N. E. Cappelletti · L. M. Tatone
Consejo Nacional de Investigaciones Científicas y Técnicas, Godoy Cruz 2290, (C1425) Autonomous City of Buenos Aires, Argentina

N. E. Cappelletti
Grupo de Investigación en Geología Ambiental, Universidad Nacional de Avellaneda-Comisión Investigaciones Científicas, España 350, (B1870) Avellaneda, Provincia de Buenos Aires, Argentina

J. C. Colombo
Comisión de Investigaciones Científicas, calle 10 y 526 (C1900), La Plata, Buenos Aires, Argentina

Springer

Introduction

Passive sampling is an increasingly accepted technique for the determination of dissolved pollutant concentrations in monitoring programs (Vrana and Popp, 2006; Booij et al., 2015) and also for studies of organism exposure, both in the laboratory and the field (Namiesnik et al., 2005) because it provides reliable time-averaged, freely dissolved pollutant concentrations (Vrana et al., 2014). This dissolved fraction is the most bioavailable, and time-averaged by passive sampling device (PSD) data minimizes the requirement for time-consuming, frequent sampling, and analysis in the highly variable aquatic ecosystems (Birch et al., 2001; Knowlton and Jones, 2006). On the other hand, bivalves have been traditionally used to monitor environmental pollutants including organic compounds and trace metals due to their usually high abundance, widespread distribution, and limited metabolizing capacity (International Mussel Watch-IMW- 1980; Sericano et al., 1995; Farrington and Tripp, 1995; Webster et al., 2009). Measuring the presence of organic contaminants in aquatic systems (i.e., biomonitoring) provides direct evidence of bioavailability and the chemical's potential for ecological and human health risks (Joyce et al., 2016). However, this strategy also presents some drawbacks related to the lack of adaptability of the sentinel species transported from other habitats; the possibility of partial degradation of pollutants; and the bioaccumulation kinetics affected by biological factors such as size, age, sex, reproductive stage, lipid content (Martin, 1992; Mersch et al., 1992; Colombo et al., 1995; Aguilar Martínez, 2009). In this context, one of the most significant applications of PSD equipped with semipermeable diffusion membranes, like low-density polyethylene (LDPE), is the ability to assess the truly dissolved fraction of organic contaminants (Huckins et al., 2006, Vrana et al., 2007). The biomimetic character of PSDs for environmental monitoring is related to the selective capacity of LDPE to allow diffusion of organic compounds from water to a receiving phase (usually C_{18}), which is analogous to the bioconcentration process occurring in aquatic organisms (Axelman et al., 2009). However, to compare accumulation kinetics between PSDs and organisms, it is necessary to consider also some differences in the uptake process. Although in both cases the transport of pollutants is controlled by the concentration gradient between water and either the acceptor phase or the tissues, in bivalves there is also an active filtration that forces the passage of high water volumes through efficient interchange tissues (i.e.,

gills and respiratory epithelium). Clam filtration rates are usually more than an order of magnitude higher (range: 7–60 1 d^{-1}, McMahom, 1983) than PSD sampling rates (0.19–0.89 1 d^{-1}, Petersen, 2015). In addition, food is a significant route of pollutant uptake for clams; therefore, it is expected that organisms have higher accumulation rates and tend to reach equilibrium faster than PSDs (Herve et al., 1991; Hoflet and Shea, 1997; Joyce et al., 2016).

Previous transplantation studies in the Rio de la Plata estuary (RLP) have confirmed the ability of *Corbicula fluminea* to accumulate PCBs (Colombo et al., 1995) and metals (Bilos et al., 1998) but there are no data on PSDs; in fact, worldwide, there are few comparative experiences of mussels or other sentinel organisms with passive sampling techniques (Booij et al., 2006; Joyce et al., 2016). Because of their hydrophobic nature, PCBs predominantly sorb to the hydrophobic portion of sediments (Ghosh et al., 2003), basically the organic carbon fraction (Jonker and Koelmans, 2002). Thus, most of these compounds accumulate in sediments and biota (i.e., lipids), while their equilibrium partitioning in water is at ultra-trace levels, resulting in very challenging analytical schemes to detect these pollutants in the dissolved phase (Arnot and Gobas, 2006).

In this study, we evaluate the performance of passive water samplers to measure the bioavailable fraction of pollutants compared to sentinel clams. Specifically, we study the accumulation kinetics of hydrophobic organic compounds such as polychlorinated biphenyls (PCBs) and two metals, one from predominantly anthropogenic sources such as copper (Cu), and another redox-sensitive, from chiefly natural sources like manganese (Mn), in both integrative sampling matrices along a 63-day exposure experiment.

Materials and methods

Study area

The Rio de la Plata (RLP) is after the Amazon, the second largest river basin in South America with one of the largest coastal plains and open estuarine zone in the world (300 km long and 30–220 km wide, Derisio et al., 2013). With a surface of 35,000 km^2, a huge drainage basin (more than 3 million km^2), and freshwater runoff (16,000–28,000 m^3/s), it receives about 160 million ton y^{-1} of particulated matter from temperate and tropical regions of South America (Moreira et al., 2013). The first 80 km along the Argentinean coast are heavily populated

(from Buenos Aires to La Plata City) supporting one-third of the total Argentinean population (~40 million) and constitute a major source of anthropogenic contaminants in the region (Barra et al., 2002). Earlier studies indicated that hydrophobic hydrocarbons, PCBs, and other xenobiotics are daily discharged to the estuary via either small tributaries or untreated effluents (Carsen Pittaluga, 2002; Robers, 2016). Pollutants associated with the abundant particulate load of the system enter the detritus food web and accumulate in bivalves and fish (Colombo et al., 1995). The sampling site selected for this work (Fig. 1) is strongly impacted by Sarandí, Santo Domingo, and Jiménez streams, the Matanza–Riachuelo river, several urban–industrial effluents, and the outfall of a local sewage treatment plant at Berazategui, Buenos Aires.

Sampler design

The PSD design is similar to the Chemcatcher® (Vrana et al., 2007, 2010), consisting of a 52 mm × 16 mm × 1 mm polypropylene hollow cylindrical body equipped with a receiving phase for organic compounds (membrane C18-3M Empore Disk 47mm) or metals (membrane Chelating-3M Empore Disk 47mm). Receptor phases were covered in both sides by either a diffusion-limiting membrane of low-density polyethylene, for the C18 phase (LDPE, 6.4 ± 0.1 Å pore size) or cellulose acetate for the Chelating phase (CA, 0.45 μm pore size). PSDs were previously calibrated in a continuous flow model exposed with both distilled and RLP water (0.5 l h^{-1}, 32 r.p.m); the accumulation kinetics of various organic pollutants and trace metals were determined considering different competing organic phases (particles and organic matter) and modeling-specific sampling rates for target analytes (Morrone et al., 2015 and Morrone, 2017; Supplementary material).

Passive sampler preparation

The conditioning of passive samplers was carried out based on the international protocols of passive water sampling (EPA 3535A SPE). A C18 disk was mounted in a 1000-ml glass filtration equipment, rinsed with 1:1—ethyl acetate:dichloromethane—and conditioned with both methanol and double-distilled water. Finally, 5 μl of a mixture solution of PCBs 30, 119, and 207 (5 μg ml) was added to each membrane as performance reference compounds (PRCs). On the other hand, the chelating disk was rinsed with deionized water in a 250-ml polypropylene filtration equipment, and then, the membrane was conditioned with HNO$_3$. The process ends with the conversion to an ammoniacal form adding an ammonium acetate buffer pH 5.3 and a final elution with deionized water.

PSDs and clams deployment

The Asiatic clam *Corbicula fluminea* was used as a sentinel organism for the encagement experience. Bivalves
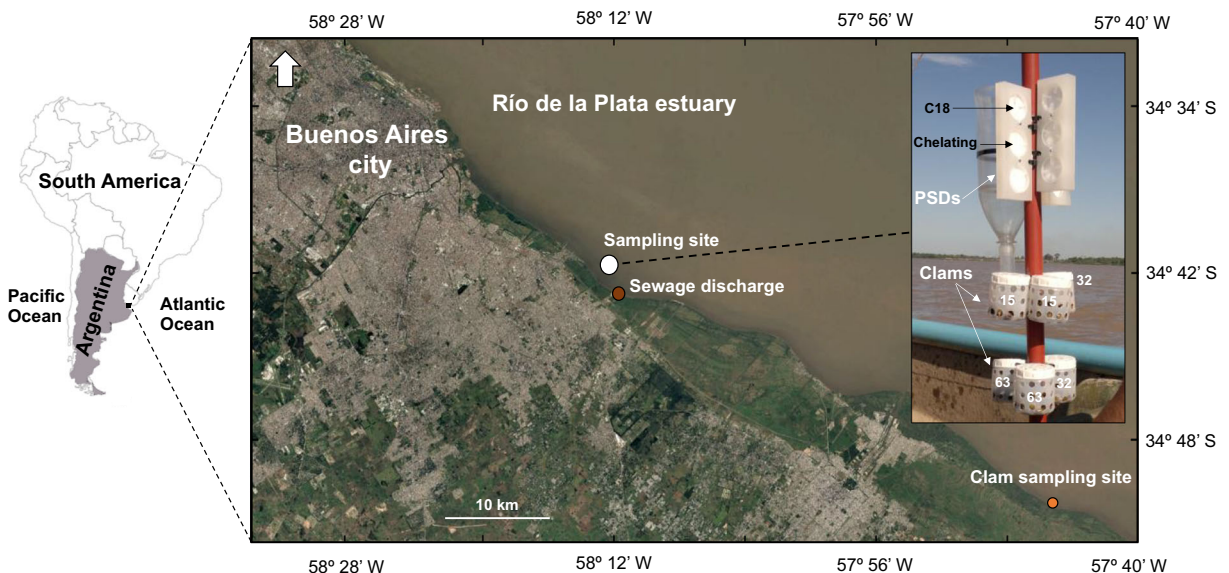
Fig. 1. Study area and deployment site on the Buenos Aires metropolitan coast, downstream of the sewage discharge. The relatively unpolluted sampling site of transplanted clams is also indicated

were collected on March 16, 2015 during low tide in a relatively non-polluted area from the RLP estuary, La Balandra, Buenos Aires. Organisms transferred to the laboratory (n=148) were depurated 72 hours in tap water (PCB and metal concentrations fell below detection limits) in order to clear the gut contents and measured (umbo height, anterior–posterior length, and dorso-ventral length) and weighted before deployment. Clams were classified into 3 arbitrary sizes based on the anterior–posterior length: large (> 30 mm; $n = 20$), medium (20–29 mm; $n = 48$), and small (<20 mm; $n = 80$). The field experiment was carried out with the more abundant small clams which were caged in 6 plastic containers (10 clams in each one); 20 clams were reserved as the control sample for the initial time of the experience (time cero). The deployment strategy consisted of a central 3/4-inch diameter PVC tube carrying six clam containers, three C18-PSD, and three chelating PSD (Fig. 1). The tube was attached to a mooring line stretched between an anchor and a subsurface buoy to keep the samplers in a stable vertical position throughout the sampling period. After deployment (time 0) and taking into account that 14–30 days is the typical time scale for deployment of the devices in the field (Colombo et al., 1995; Vrana and Popp, 2006), the site was visited at 15, 32, and 63 days for recovery of two clam containers, one C18-PSD, and one chelating PDS, with the simultaneous collection of a spot water samples in 4-l amber glass bottles and *in situ* measurement of water quality parameters (temperature, dissolved oxygen, conductivity, turbidity, and pH) with portable multiparameter probe (Horiba U-52-10). Bivalves were transferred to the laboratory immediately after collection, dissected with scalpel, and drained from excess water (opened on their ventral edge over tissue paper). Pooled fresh tissues (20 individuals, ~10 g) were homogenized in glass jars with a steel spatula and three sub-samples were separated for moisture content determination (~1 g; 24 h at 100°C), metals analysis (~2 g), and extraction of organic pollutants (~7–8 g wet weight).

Chemical analysis of passive samplers, water, and clams

Water samples

Analysis of dissolved PCBs in spot samples was performed by solid phase extraction (SPE) in a serial system composed of a sample reservoir, a filter holder containing a glass fiber filter to retain suspended particulated material (S. Munktel MGF 90 mm, 0.7 μm pore size), and an hydrophobic C18 phase (Bakerbond Speedisk™ C18) connected to a vacuum pump. After rinsing and conditioning the C18 phase with dichloromethane–ethyl acetate and methanol, respectively, approximately 2–3 l of water was filtered. After addition of surrogate standards (PCB103, PCB198, Phenanthrene-d10 and Chrysene-d12, Absolut Standard), the C18 phase was eluted with 5 ml of dichloromethane/ethyl acetate in a glass tube. The organic extracts were dried (Na$_2$SO$_4$) and concentrated to a final volume of 500 μl by evaporation under nitrogen in a thermostatic bath at 40 °C.

For metal analysis, water samples were filtered through a cellulose acetate membrane (Gamafil 47 mm, 0.45 μm pore size) in a 250-ml polypropylene filtration equipment and then placed in 60-ml acidified plastic bottles (HNO$_3$, pH 2), preserved at 4°C until analysis.

PSDs

After the exposure period, the PSDs were transported to the laboratory wrapped in aluminum foil and refrigerated. In the laboratory, they were disassembled and the receiving phases were processed according to the corresponding protocols. Analytes retained in the C18 phase were extracted, with a previous addition of internal standards (PCB103, PCB198, Phenanthrene-d10 and Chryeno-d12; Absolut Standard), by elution with ethyl acetate and dichloromethane under vacuum. Organic extracts were dehydrated (Na$_2$SO$_4$) and concentrated to 500 μl by evaporation under nitrogen flow at 40 °C. Metals retained in chelating membranes were extracted by elution with nitric acid 3 M (20 ml) under a slight vacuum in 60-ml plastic bottles which were kept at 4 °C until analysis.

Truly/apparent dissolved concentrations and speciation in water

In this study, truly dissolved (or freely), apparent dissolved, and particulate pollutants were discriminated in a strictly operational way. The particulate fraction retained in the fiberglass filters was not considered in this paper. PCB concentrations in the filtrate were considered as the so-called apparent dissolved concentration (AC), because it includes the truly dissolved phase but also compounds adsorbed to dissolved organic

carbon (DOC) that is retained on the C18 disk, thus artificially enhancing the PCBs concentrations (Li and Lee, 2000; Brown and Peake, 2003). The truly dissolved contaminant phase (TD) was characterized exclusively by the fraction collected by the PSDs, whose selectivity is controlled by the pore size of diffusion membranes (LDPE). LDPE polymer is described as nonporous material, but random thermal motions of their chains form transient cavities with maximum diameters of approximately 10 Å. Hydrophobic solutes are essentially dissolved by the polymer because of the small size and dynamic character of polymer cavities. This size limitation excludes large molecules as well as those that are adsorbed on colloids or humic acids. Only truly dissolved and non-ionized contaminants diffuse through the LDPE membrane can be separated by the sampler (Esteve-Turrillas et al., 2008). In the case of metal speciation in water, it can be conceptually described as a series of equilibrium reactions between hydrated or free metal ions $(M(H_2O)_x{}^{2+})$, organic and inorganic complexes (labile and non-labile) of small (i.e., $M(CO_3)_2{}^{-2}$), and large macromolecules (i.e., fulvic and humic acids) and finally the fraction associated with suspended particles (Knutsson, 2013). Accumulation in the passive sampler is based on the diffusion of metal species across the cellulose acetate membrane and accumulation in the receiving phase; therefore, measurements exclude metals sorbed to suspended sediments and those forming complexes unable to dissociate in the time required to pass through the diffusion layer (Allan et al. 2010).

Clams

The homogenized clam tissues (7–8 g wet weight) were dried with sodium sulfate (1.5–2 times), fortified with surrogate standards (PCB103, PCB198, Phenanthrene-d10 and Chrysene-d12, Absolute Standard), and extracted with dichloromethane under ultrasound (4 times × 15 min). The supernatants separated by centrifugation were combined in glass tubes, concentrated to 5 ml and split for the determination of total lipids (gravimetry of a 200-µl aliquot evaporated to dryness) and PCB analysis. This included the partial removal of lipids by sulfuric acid treatment with further purification of the concentrated petroleum ether supernatants by neutral silica gel column chromatography (1 g, JTBacker 60–200 µm) eluting the PCB fraction with 5-ml petroleum ether.

Tissue subsamples for metal analysis (~ 2 g wet weight) were calcined at 400-450 °C and digested with aqua regia (3 : 1 HNO_3 and HCl), and H_2O_2 at 100 °C on a heating plate. After cooling, solutions were transferred to polyethylene tubes and centrifuged (4000 rpm, 20 min) to remove solids. Extracts were diluted to 25 ml with deionized water and stored in polyethylene bottles.

Instrumental analysis

PCBs were analyzed by HRGC (Agilent 7890) with a DB-5 capillary column (30 m × 0.32 mm, 0.25 mm) and an electron capture detector. Details of chromatographic conditions and temperature programs are given in the Supplementary material. The external standard method was used to determine 41 PCB congeners: tri-CBs: 17, 18, 31/28, 33/53; tetra-CBs: 44, 49, 52, 70, 74; penta-CBs: 87, 95, 99, 101, 110, 118; hexa-CBs: 128, 132/105, 138, 151/82, 153, 156,158, 169; hepta-CBs: 170, 171, 177, 180, 183, 187,191; octa-CBs: 194, 195, 199, 205; nano-CBs: 206, 208; and deca-CBs: 209. Detection limits for PCBs (3 : 1 signal to noise) ranged from 0.1 to 3.0 ng g^{-1} dry weight for clams and PSDs and 0.04 ng l^{-1} for water samples. Procedural blanks—one for every batch of seven samples—were below detection limits, and surrogate recoveries ranged between 63 and 70% for PCBs (Supplementary material). A cod liver oil standard reference material (SRM 1588) purchased from the National Institute of Standards and Technology (NIST) were analyzed for QA/QC purposes. Analysis of the NIST reference material indicated that the PCB analytes were quantified to within 15% of their certified concentration (Table 2S, SM). Method precision evaluated through repeated analysis of an internal reference fish material prepared with Rio de la Plata *P. lineatus* muscle averaged 16% ($n = 5$)

Metal concentrations were determined using a Thermo Elemental Solaar M5 atomic absorption spectrophotometer equipped with a deuterium background corrector using a GF95 graphite furnace with autosampler. Procedural blanks were negligible. The precision of trace metal determinations as the relative standard deviation (RSD) tested through the analysis of 5 duplicate samples was below 10%. The recovery method was tested through a triplicate analysis of a Certified Reference Soil (CRM020-050 RTC). The average recoveries ranged from 97.3±5.3 for Mn to 99.7± 2.8% for Cu.

Data analysis

Statistical analysis was carried out using Xlstat (Addinsoft.). Data were expressed as mean ± standard deviation. The kinetic analysis was performed by linear regression and slopes were compared by ANCOVA using a significance level $p < 0.05$. PCB concentrations in solid samples are expressed in dry weight (dw), C18-normalized (1 g of C18 membrane contains 0.2 g of C18, Verhaar et al., 1995), or lipid-normalized (Lc) weight as indicated. Metal concentrations in membranes and clams are expressed in dry weight (dw).

Results and discussion

PCBs

Total concentrations of PCB measured in water, PSDs, and clams are presented in Table 1 and Table 3S of Supplementary material. PCB concentrations in spot water samples (35–41 ng l^{-1}) are comparable with previous reports for the area (46–50 ng l^{-1}; Janiot, 2001; Colombo et al., 2001), higher than other moderately contaminated sites such as Galveston Bay, EEUU (0.49–8.5 ng l^{-1}; Howell et al., 2010), but several orders of magnitude lower than the highly polluted Hudson River, EEUU (511–1890 ng l^{-1}; U.S.EPA, 2019). Compared to Canadian water quality guidelines (CWQG) for protection of aquatic life (1 ng l^{-1}; CCREM, 1987), PCB concentrations in RLP were 35 to 41 times higher. According to EPA chronic hazard value with a continuous concentration criterion (CCC) for PCB (14 ng l^{-1}; U.S.EPA 2019), RLP concentrations were about 3 times higher. In the PSDs, PCB accumulation was linear over the exposure period (both in dry weight or C18 content basis; Fig. 2). Using laboratory-calculated sampling rates (Supplementary Material), the truly dissolved PCB concentration was 4.0±6.9 ng l^{-1} accounting for ~13 % of PCB concentration in the apparent dissolved phase from filtered spot water samples. Our freely dissolved PCB concentrations are comparable to those reported in New Bedford Harbor (1.40–16.9 ng.l^{-1}; Hofelt and Shea, 1997) and higher to that observed in the Naval Station Newport, EEUU (0.11–0.17 ng l^{-1}; Burguess et al., 2015). PCB concentrations in *Corbicula fluminea* ranged from 1622 to 3561 ng.g^{-1} Lc (144 to 263 ng.g^{-1} dw) throughout the experiment. These results are in the lower range of previously reported

Table 1 PCB concentration in the apparent and truly dissolved phase, accumulation mass in PSD (Mb$_{C18}$) and clams (Lc). Metal concentration measured in spot sampling and passive sampling, accumulation mass in PSD (chelating membrane) and clams during the experience course

Days	PCBs				Copper				Manganese			
	Apparent concentration ng.l^{-1}	Truly dissolved	PSD–mass C18 ng.g^{-1}	Clams (Lc)	Spot sampling µg.l^{-1}	Passive sampling	PSD–mass chelating µg.g^{-1}	Clams (dw)	Spot sampling µg.l^{-1}	Passive sampling	PSD–mass chelating µg.g^{-1}	Clams (dw)
0	46.8	-	-	800	2.46	-	-	3.81	-	-	-	6.58
15	46.1	4.0	108	1622	5.09	2.29	1.80	5.13	0.36	23.5	32.7	9.74
32	34.8	4.9	257	3032	3.16	4.16	3.50	6.09	0.36	31.3	43.6	12.80
63	40.8	6.9	606	3561	3.50	8.02	6.54	6.42	20.8	93.6	130	17.83
Average	42.1	5.27	323	2253	3.55	4.82	3.95	5.36	7.17	49.47	68.77	11.74
Std Dev.	5.57	1.48	255	1268	1.11	2.92	2.40	1.17	11.80	38.42	53.31	4.79

Fig. 2 Kinetics of accumulation of PCBs in the PSDs and clams expressed as dw (above) and normalized by lipids and carbon contents (Lc and C18; below)
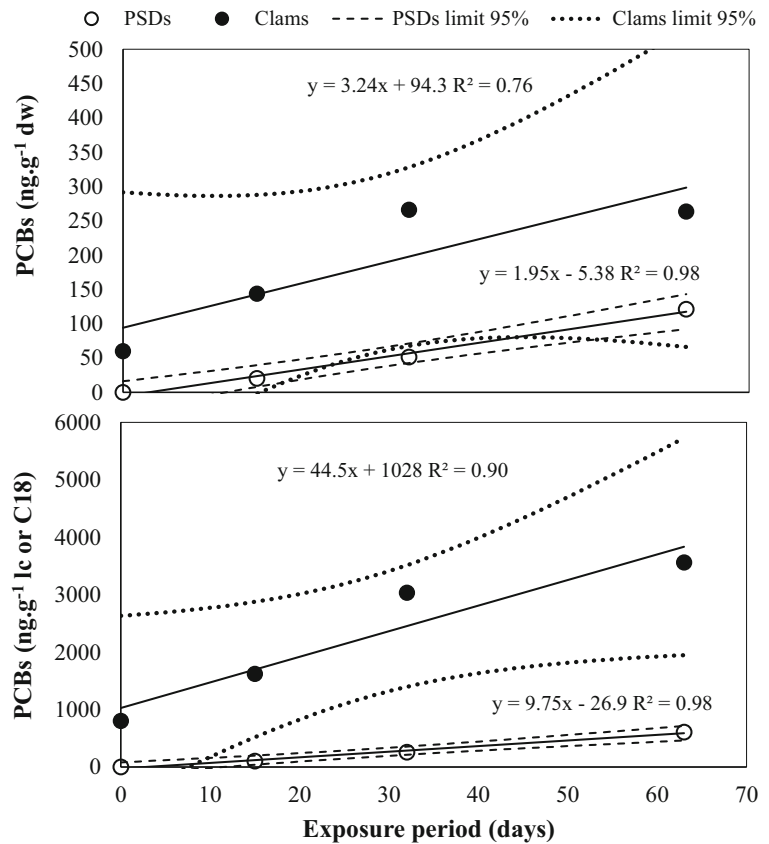
concentrations for the same clam species transplanted to the impacted La Plata harbor which attained final PCB concentrations of 2800–9200 ng.g^{-1} Lc after 68 days of exposure (Colombo et al., 1995). This PCB reduction in the clams is consistent with the general long-term decrease of PCBs in sediments, settling particles, and fish reported for this area (Colombo et al., 2018). The bioaccumulation trend of PCBs in the clams shows an apparent loss of linearity at day 63 which is consistent with previous transplant results which stabilized after 68 days (Colombo et al., 1995) suggesting equilibrated uptake and elimination rates (steady state). In absence of significant metabolism by the clams, pollutant elimination is chiefly through the exchange with the water phase, as have been reported for other bivalves (Björk and Gilek, 1997). Therefore, the equilibrium stabilization is controlled basically by physiological factors, environmental conditions, and pollutant concentration gradients (Moy and Walday, 1996; Arnot and Gobas, 2006). Our results are also comparable to absorption tests of PCBs performed with *Chamaelea gallina*, which showed comparable accumulation kinetics for

30–60 days' exposure in flow through channels (Rodriguez-Ariza et al., 2003). The time–trend slopes of PCB accumulation in clams (3.2±1.3 and 45±10 in dry and lipid basis, respectively) were 3–4 times higher (ANCOVA; $p<0.05$) than those of PSDs (1.9±0.14 and 9.8±0.69 in dry and C18 basis, respectively), reflecting enhanced bioaccumulation through active filtration and PCB uptake from retained particles (see the relative composition of PCBs section), emphasizing the significance of the alimentary route.

Relative composition of PCBs

Homolog profiles in the apparent dissolved phase from spot water samples were dominated by tri, tetra, penta and hexa-CB (21%, 31%, 17% and 15% of total PCBs, respectively) with a prevailing contribution of congeners tri-CB 33/55/20 (14%), tetra-CB 44 (18%), tetra-CB 74 (17%), penta-CB 99 (7%), hexa-CB 151/82(5%), penta-CB 101(4%), and hexa-CB 128/167 (2%). Conversely, in PSDs, tri-CBs have limited detection, with predominance of tetra and penta chlorobiphenyls (21

and 50% of total PCBs, respectively) and congeners tetra-CBs 44 (9%) and 74 (7%), penta-CBs 101 (27%), 99 (9%), and 87–115 (5%). This pattern is consistent with those reported for urban rivers in Shanghai, China where penta-CBs contributed more than 70% of total PCBs (Wang, 2016). The PCB pattern in clams exhibited a shift toward heavier penta- and hexa-CBs (43 and 41% of total PCBs, respectively) with a predominance of penta-CB congeners 118 (17%), 101 (9%), and 110–77 (7%), and hexa-CBs 153 (19%), 138 (8%), and 149–123 (6%). Similar homolog patterns dominated by penta and hexa-CBs (25–32% and 50–51% of total PCB) were described for previous transplant experiences in the Río de la Plata (Colombo et al., 1995). These clam PCB homolog profiles resemble more those reported for suspended particulate matter (Colombo et al., 1995) and settling particles (Colombo et al., 2005), dominated by penta-CBs (25–33%) and hexa-CBs (51–45%), than the true dissolved PCB trace found in this study. This suggests that particulated material is likely the most significant source of PCBs for the clams. A previous, biodynamic modeling study of *C. fluminea* reported that the aqueous uptake of PCBs was almost equally important to dietary uptake, with a similar aqueous and sediment absorption efficiency (~ 20 % each; Mcleod, 2008). Additionally, PCB accumulation and clearance depend on chlorination degree and congener structure thus affecting PCB patterns. In bivalves, clearance half-lives increase with chlorination degree and thus with the hydrophobicity and lipophilicity of each congener (Rodriguez-Ariza, 2003). Finally, compound uptake via fine filterable particles or colloids (<1–10 µm, McMahom, 1983) can also contribute to overlap PCB profiles in the clams, shifting the pattern to higher chlorinated congeners compared to the PSD signal which includes only the truly dissolved phase.

Individual congener uptake kinetics

Nine to three hexachlorobiphenyl PCB congeners well represented in both PSDs and clams were selected to model the accumulation kinetics of individual congeners (tetra-CBs 44 and 74; penta-CBs 87–115, 99, 101, 110 and 118; and hexa-CBs 149 and 153). In PSDs, regression analysis indicated that all of PCB congeners except penta-CB 101 showed statistically significant ($p<0.05$) positive slopes decreasing with the number of chlorine substituents (0.86 ± 0.19, 0.41 ± 0.08, and 0.20 ± 0.09 for tetra-,

penta-, and hexa-CB, respectively), and thus show an inverse relationship with congener hydrophobicity (Figs. 3 and 4). Consistently, in a freshwater calibration assay with Chemcatcher®, Petersen (2015) found that accumulation slopes of PCB congeners 52, 101, and 153 ranged between 0.20 and 0.48 decreasing with higher chlorination. Literature data on contaminant uptake by semipermeable membrane devices provides some insight on the observed slopes. The uptake rate is controlled by two limiting barriers: (1) the boundary layer, a film of stagnant water and (2) the permeability or resistance to transport inside the membrane. For more hydrophobic compounds (log $K_{OW} > 4.5$), fewer molecules are in the water phase and consequently the mass crossing the boundary layer would be smaller while the transport abilities in the receiving phases are less affected by increasing hydrophobicity (Booij et al., 2007).

In clams, regressions of congener concentrations versus time were statistically non-significant due to the previously mentioned stabilization by the end of the exposure period. Despite this situation, the slope analysis enables some interesting observations. In contrast to PSDs, the accumulation slopes for tetra-, penta-, and hexa-CB increased with chlorination (0.42 ± 0.51, 3.77 ± 2.65, and 4.90 ± 8.02, respectively), and thus showed a direct relationship with congener hydrophobicity (Fig. 3). The uptake kinetics of PCBs in clams is influenced by the dietary incorporation of solid particulate material, colloids, and dissolved organic matter which are enriched in higher chlorinated congeners. This has already been hypothesized in previous *Corbicula fluminea* studies (Colombo et al., 1995) which suggested that PCB bioaccumulation in the clams depended mainly on the ingestion of solid particulate material with a higher affinity for superhydrophobic molecules. The ratios between accumulation slopes in clams and PSDs were used to compare both kinetics. As shown in Fig. 4, the ratios ranged between 0.4 and 27, increasing with hydrophobicity (Log K_{OW}). This behavior indicates that PSDs have a greater affinity for the most soluble fraction of PCBs; therefore, the uptake rate is controlled by diffusion in the water boundary layer (WBL; Rusina et al., 2010). On the other hand, according to Mcleod (2008), *Corbicula fluminea* takes up approximately 45 % of its PCB body burden through sediment ingestion facilitating the accumulation of higher chlorinated and more hydrophobic congeners. Additionally,
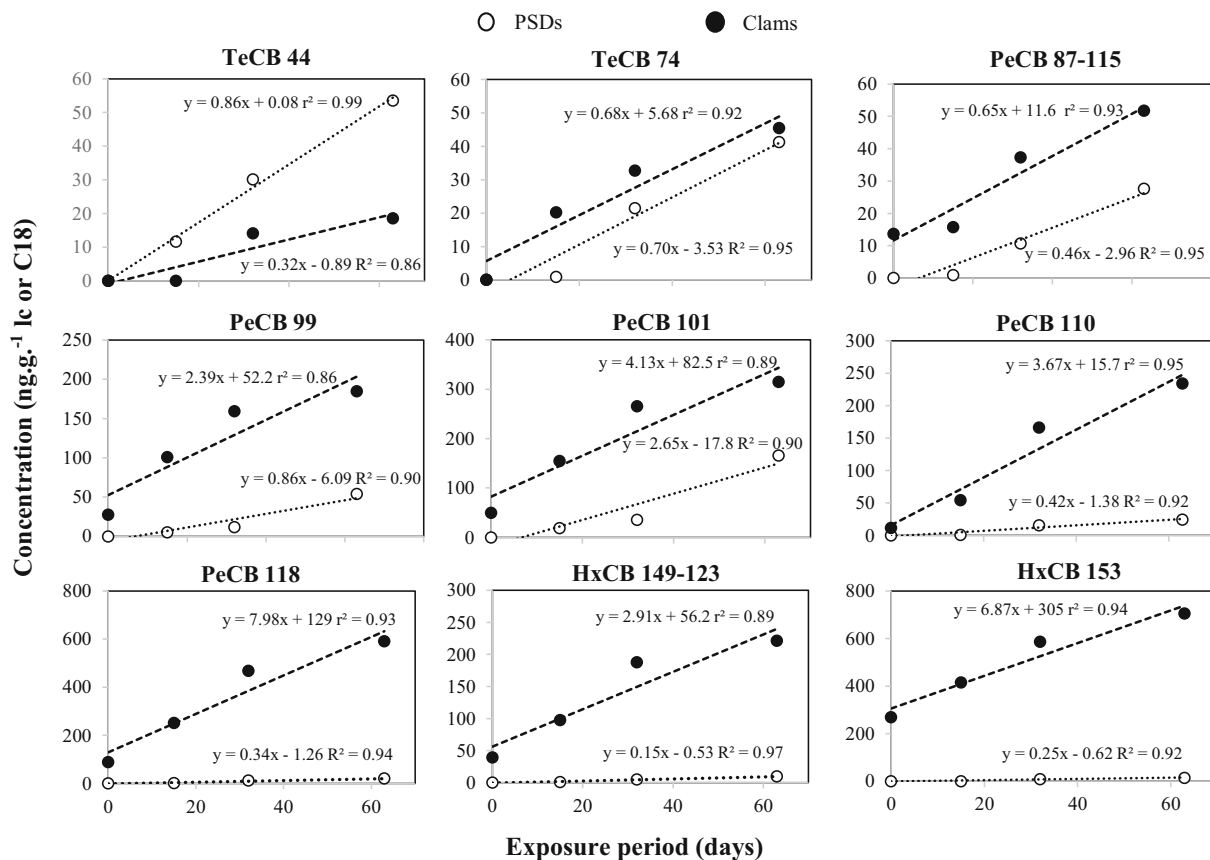
Fig. 3. Individual accumulation kinetics (ng g^{-1} dw) of selected PCB congeners in clams (black circles) and passive samplers (open circles)

in actively ventilating organisms, diffusion may be increased by the convective currents produced by the clams which reduce the boundary layer barrier (Huckins et al., 2006). Both processes would enhance the uptake of more hydrophobic, higher chlorinated congeners in the clams.

Metals

Cu and Mn concentrations measured in water, PSDs, and clams are presented in Table 1. Dissolved Cu concentrations measured in spot water samples (2.4–5.0 µg l^{-1}) are consistent with previous measurements carried

Fig. 4. Accumulation slope ratios between clams and PSDs for selected PCB congeners as a function of octanol–water partition coefficient (Log K_{ow})
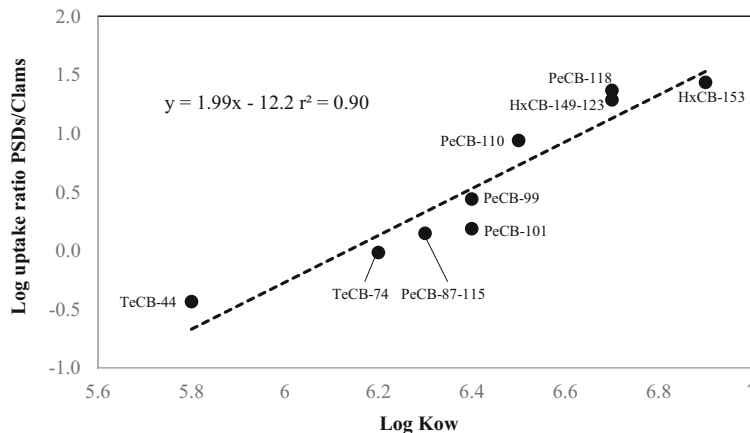
out in the RLP (6.4 µg l^{-1} ; Janiot, 2001; 8.7 µg l^{-1}; Colombo et al., 2001) and somewhat lower to results from PSDs (2.3–8.0 µg l^{-1}). Cu concentrations in RLP were approximately 2 times higher than CWQG (2 ng l^{-1}; CCREM 1987) and these were below CCC (6 to 22 ng l^{-1}; U.S.EPA, 2019) considering a hardness range between 40 and 159 mg l^{-1} CaCO$_3$ and pH 7.5.

In contrast to PCBs, a better agreement between PSD and spot water is expected for metals, especially if the predominant Cu species are free ions or Cu associated to labile organic and inorganic complexes. The mass of Cu accumulated in PSDs throughout the experience displays a significant linear trend ($p<0.05$), with concentration ranging between 1.8 and 6.5 µg g^{-1}. A similar, but no significant linear trend, was exhibited in organisms, with concentrations ranging from 3.8 to 6.4 µg g^{-1}. Although the final concentration in the clams was very similar to that of PSDs (6.4 vs. 6.5 µg g^{-1}, respectively), the passive sampler slope is 3 times higher (0.10 ±0.01 vs. 0.04±0.05 in the clams, Fig. 5). High Cu concentrations in control clams are mainly due to low clearance rates as has been reported for *Corbicula fluminea* evaluated in laboratory exposure tests (Croteau, 2005). This agrees with previous observations in RLP *Corbicula fluminea* whose Cu concentrations were among the highest reported for freshwater bivalves (Bilos et al., 1998). Therefore, PSDs show a higher copper accumulation efficiency compared to clams which have already high Cu initial concentrations.

Dissolved Mn concentrations obtained in spot water sampling (0.36–20.8 µg l^{-1}) were lower than PSD values (24–94 µg l^{-1}). Such discrepancy and the variability of Mn concentrations obtained in the spot sampling (64%) might reflect the variability associated to redox changes in the water. The phase distribution of redox-sensitive elements such as Mn is controlled by physical, chemical, and biological processes. Briefly, under reducing conditions, Mn is relatively soluble and geochemically stable as Mn(II), whereas under less reducing regimes, it is oxidized to Mn(III) and Mn(IV) and thus transferred to the particulate phase (Solomons and Förstner, 1984). It should be noted that in this polluted RLP area, redox conditions are very variable according to the impact of the effluent's plumes (dissolved oxygen: 5.5–10.1 mg l^{-1}) and the possible influence of anoxic bottom sediments. The anaerobic–suboxic characteristics of bottom sediments facilitate the evasion of Mn to the dissolved phase through reductive dissolution of Mn oxides, being a major driving factor explaining the low Mn concentrations in bottom sediments of this polluted site (Tatone et al., 2020). Conversely, Mn precipitation/sedimentation under aerobic conditions in the water column would also occur (Ferro, 2003; Yakushev et al., 2009). This could explain the lower Mn concentrations measured in the spot sampling of surface water. This redox-dependent variability of Mn can be effectively integrated by PSDs. Overall, Mn concentrations in RLP were more than an order of magnitude below Canadian guidelines (430 ng l^{-1}; CCME, 2019).

The concentration of Mn in PDS and clams increased linearly over time ($p<0.05$), ranging from 33 to 130 µg gr^{-1} dw and 6.6 to 18 µg gr^{-1} dw, respectively. The accumulation slope in PDS (2.0±1.3) was one order of magnitude higher than those exhibited by clams (0.18 ±0.03). Mn is considered to be poorly bioaccumulated in Asian Clams (Shoults-Wilson et al., 2010). Previous studies have corroborated that Mn content in soft clam
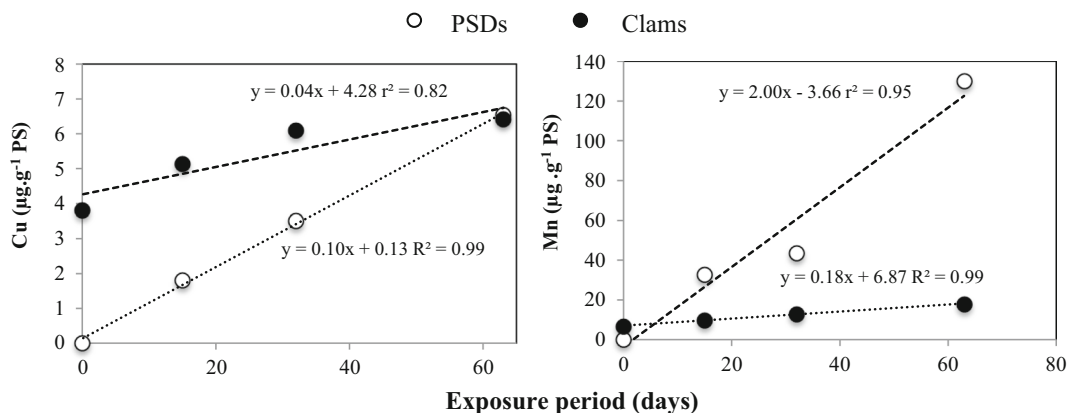

Fig. 5. Copper and manganese accumulation kinetics in the PSDs and clams

tissues is independent of their body size and tends to remain constant throughout their life cycle (Bilos et al., 2009). Moreover, Zhao (2016) reported that *Corbicula fluminea* incorporates Mn to the shells, depending mainly on the competition with Ca^{2+} which has the same transport routes. The consequence is a low Mn accumulation in soft tissues suggesting that Asiatic clams are not suitable for Mn biomonitoring. In contrast, PSDs demonstrated excellent performance for monitoring dissolved manganese avoiding the correction of data by biological factors or by redox variations.

Conclusions

To the best of our knowledge, this is one of the few studies reporting the simultaneous accumulation kinetics of PCBs and metals in PSDs and caged bivalves (Asiatic clams) along a 63-day exposure experience. PSDs displayed linear accumulation kinetics of pollutants along the deployment. The truly dissolved PCB phase characterized by the fraction collected by the PSDs, accounted for ~13 % of the apparently dissolved PCB phase as measured in the filtered spot water samples. PSDs accumulated more efficiently the most soluble tetra- and penta-CBs through diffusion in the water boundary layer whereas Asiatic clams accumulated chiefly the most hydrophobic PCBs reflecting significant uptake through food particles. This resulted in higher accumulation slopes in the clams compared to PSDs but with an apparent loss of linearity at day 63 suggesting that steady state has been attained at the end of the experience. In contrast to PCBs, dissolved Cu concentrations from PSDs and spot water samples resulted more comparable suggesting that Cu speciation as free ions and labile organic and inorganic copper complexes, was adequately integrated by passive sampling. However, for Mn, both monitoring devices showed dissimilar results possibly reflecting the redox sensitivity of this metal and consequent high variability in the water column affected by anthropogenic discharges and anoxic sediments. The accumulation kinetics of both metals was 3.3–11 times higher in PSDs reflecting the different metal selectivity of clams which accumulate Cu with high initial concentrations and have a poor bioaccumulation of Mn.

References

Allan, I. J., Booij, K., Paschke, A., Vrana, B., Mills, G. A., & Greenwood, R. (2010). Short-term exposure testing of six different passive samplers for the monitoring of hydrophobic contaminants in water. *Journal of Environmental Monitoring*, 12(2010), 696–703.

Aguilar Martinez, R. (2009). *Desarrollo del muestreador pasivo chemcatcher para la monitorización y cuantificación mediante GC-ICP-MS de mercurio y compuestos organoestánnicos en medios acuáticos*. Tesis doctoral: Universidad de Madrid.

Arnot & Gobas, (2006). A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Environmental Reviews 14: 257–297* (2006) doi: 10.1139/A06-005.

Axelman, J., Naes, K., Naf. C., Broman, D. (2009). Accumulation of polycyclic aromatic hydrocarbons in semipermeable membrane devices and caged mussels (mytilus edulis l.) in relation to water column phase distribution. *Environmental Toxicology and Chemistry*, Vol. 18, No. 11, pp. 2454–2461, 1999.

Barra, R., Colombo, J.C., Gamboa, N., Eguren, G., Jardim, W. (2002). Regionally based assessment of persistent toxic substances: Eastern and Western South America Regional Report, Argentina, Bolivia, Brazil, Chile, Ecuador, Paraguay, Peru, Uruguay. *United Nation Environment Program, Chemicals*, UNEP-GEF, 101p.

Bilos, C., Colombo, J. C., & Rodriguez Presa, M. J. (1998). Trace metals in suspended particles, sediments and Asiatic clams (Corbicula fluminea) of the Río de la Plata Estuary, Argentina. *Environmental Pollution, 99*, 1–11.

Bilos, C., Colombo, J. C., Skorupka, C. N., Demichelis, S. O., & Tatone, L. M. (2009). Size-related trace metal bioaccumulation in Asiatic clams (Corbicula fluminea) from the Rio de la Plata estuary, Argentina. *International Journal of Environment and Health, 3*(4), 390.

Birch, G. F., Taylor, S. E., & Matthai, C. (2001). Small-scale spatial and temporal variance in the concentration of heavy metals in aquatic sediments: a review and some new concepts. *Environmental Pollution, 113*, 357–372.

Booij, K., Smedes, F., Van Weerlee, E. M., & Honkoop, P. J. C. (2006). Environmental Monitoring of Hydrophobic Organic Contaminants: The Case of Mussels versus Semipermeable

Membrane Devices. *Environmental Science & Technology,* *40*(12), 3893–3900.

Booij, K., Vrana, B., & Huckins, J. M. (2007). *Chapter 7:Theory, modelling and calibration of passive samplers used in water monitoring* (pp. 141–169). Amsterdam: Passive Sampling Techniques in Environmental Monitoring. Elsevier.

Brown, J. N., & Peake, B. M. (2003). Determination of colloidally-associated polycyclic aromatic hydrocarbons (PAHs) in fresh water using C18 solid phase extraction disks. *Analytica Chimica Acta, 486*(2003), 159–169.

Booij, K., Robinson, C. D., Burgess, R. M., Mayer, P., Roberts, C. A., Ahrens, L., Allan, I. J., Brant, J., Jones, L., Kraus, U. R., Martin, M. J., Peter, L., Jördis, L., & Petersen, D. (2015). Passive sampling in regulatory chemical monitoring of non-polar organic compounds in the aquatic environment. *Environmental Science & Technology, 2016*(50), 3–17.

Burguess, R.M., Lohman, R., Schubauer-Berigan, J.P., Reitsma, P., Perron, M., Lefkovitz, L., Cantwell, M. (2015). Application of passive sampling for measuring dissolved concentrations of organic contaminants in the water column at three marine superfund sites. *Environmental Toxicology and Chemistry*, Vol. 34, No. 8, pp. 1720–1733, 2015

Carsen Pittaluga, A. E. (2002). Aportes de contaminantes y sedimentos al Río de la Plata interior. *Protección ambiental del Río de la Plata y su frente marítimo: Prevención y control de la contaminación y restauración de hábitats* (PNUD/GEF RLA/99/G31).

CCREM (Canadian Council of Resource and Environment Ministers). 1987. Canadian water quality guidelines. Task Force on water quality guidelines. Ottawa, Canada.

CCME (Canadian Council of Ministers of the Environment), 2019. Canadian water quality guidelines for the protection of aquatic life: Manganese (dissolved).

Colombo, J. C., Bilos, C., Campanaro, M., Rodriguez Presa, M. J., & Catoggio, J. A. (1995). Bioaccumulation of polychlorinated biphenyls and chlorinated pesticides by the Asiatic clam Corbicula fluminea; its use as sentinel organism in the Rio de La Plata Estuary, Argentina. *Environmental Science & Technology, 29*(4), 914–927.

Colombo, J.C. et al. (2001). Aspectos biogeoquimicos del impacto del emisario cloacal en el ecosistema costero del Río de la Plata. Segunda parte del informe: *Las cloacas máximas y la franja costera Sur del Gran Buenos Aires. Antecedentes y contaminación actual.* Municipalidad de Berazategui.

Colombo, J. C., Cappelletti, N., Barreda, A., Migoya, M. C., & Skorupka, C. N. (2005). Vertical fluxes and accumulation of PCBs in coastal sediments of the Río de la Plata estuary Argentina. *Chemosphere, 61,* 1345–1357.

Colombo, J. C., Speranza, E. D., Astoviza, M., Migoya, M. C., Skorupka, C. N., Morrone, M., Heguilor, S., Tatone, L. M., & Bilos, C. (2018). Long-term (1970-2017) temporal trends of PCBs in fish, settling material and sediments from populated and remote sites from the Río de la Plata estuary, Argentina. *Environmental Science & Technology, 52*, 12412–12418.

Derisio, C.; Braverman, M; Gaitán, E.; Hozbor, C.; Ramírez, F.; Carreto, J., Botto, F., Gagliardini, D., Acha, M., Mianzan H. (2013). The turbidity front as a habitat for Acartia tonsa (Copepoda) in the Río de la Plata, Argentina-Uruguay . *Journal of Sea Research* Volume 85, January 2014, Pages 197-204.

Farrington, J.W., Tripp, B.W. (1995). International Mussel Watch Project. Initial implementation phase; *NOAA Technical Memorandum NOS ORCA 95; NOAA: Silver Spring,* MD, 1995.

Ferro, I. (2003). Cycling of iron and manganese in freshwater, estuarine and deep sea sediments. doctoraat in de Wiskunde en Natuurwetenschappen aan de Rijksuniversiteit Groningen, Nederland.

Herve, S., Paukku, R., Paasivirta, J., Heinonen, P., & Sodergren, A. (1991). Uptake of organochlorines from lake water by hexane-filled dialysis membranes and by mussels. *Chemosphere, 22*, 997–1001.

Hofelt, C., & Shea, D. (1997). Accumulation of organochlorine pesticides and PCBs by semipermeable membrane devices and Mytilus edulis in New Bedford Harbor. *Environmental Science & Technology, 31*, 154–159.

Howell, N., Habadi, R., & Koenig, L. (2010). Comparative distribution, sourcing, and chemical behavior of PCDD/Fs and PCBs in an estuary environment. *Chemosphere, 83*(2011), 873–881.

Huckins, J.N., Petty, J. D., Booij, K. (2006). Monitors of organic chemicals in the environment: Semipermeable membrane devices; *Springer: New York.* ISBN 978-0-387-35414-9

International Mussel Watch. (1980). *Report of a workshop sponsored by the environmental studies board Commission on Natural Resources National Research Counsil*. D.C.: Washington.

Jonker, M. T. O., & Koelmas, A. (2002). Sorption of Polycyclic Aromatic Hydrocarbons and Polychlorinated Biphenyls to Soot and Soot-like Materials in the Aqueous Environment: Mechanistic Considerations. *Environmental Science & Technology, 36*(17), 3725–3734.

Joyce, A. S., Portis, L. M., Parks, A. N., & Burgess, R. M. (2016). Evaluating the relationship between equilibrium passive sampler uptake and aquatic organism bioaccumulation. *Environmental Science & Technology, 50*(21), 11437–11451.

Knowlton, M. F., & Jones, J. R. (2006). Temporal variation and assessment of trophic state indicators in Missouri reservoirs: Implication for lake monitoring and management. *Lake and Reservoir Management, 22*, 261–271.

Li, N., & Lee, H. K. (2000). Tandem-cartridge solid-phase extraction followed by GC/MS analysis for measuring partition coefficients of association of polycyclic aromatic hydrocarbons to humic acid. *Analytical Chemistry, 2000*(72), 5272–5279.

Martin, M., (1992). California Mussel Watch: Monitoring metal and trace organic toxicants in marine waters. In: Miskiewicz, A.G. Ed., Proceedings of ae organic toxicants in marine waters. In: Miskiewicz, A.G. Ed., Proceedings of a bioaccumulation workshop: assessment of the distribution, impacts and bioaccumulation of contaminants in aquatic environments. *Water Board and Australian Marine Sciences Association.* Inc., Sydney, pp. 15±37. M.

Mersch, J., Jeanjean, A., Spor, H. y Pihan, J. C. (1992). The freshwater mussel Dreissena polymorpha as a bioindicator for trace metals, organochlorines and radionucleides. En: Ecology, biology monitoring and first applications in the water quality management, (Eds. D. Neumann, H.A. Jenner), *Fischer Verlag, New York*, NY., pp. 227-244

Mcleod, P., Luoma, S., & Luthy, R. (2008). Biodynamic modeling of PCB uptake by Macoma balthica and Corbicula fluminea from sediment amended with activated carbon. *Environmental Science & Technology, 2008*(42), 484–490.

McMahom, R. F. (1983). The MoZlusca, ecology; Wilbur, K.M., Ed.; *Academic Press:New York. Vol., 6*, 505–561.

Moreira, D., Simionato, C. G., Gohin, F., Cayocca, F., & Luz Clara Tejedor, M. (2013). Suspended matter mean distribution and seasonal cycle in the Río de La Plata estuary and the adjacent shelf from ocean color satellite (MODIS) and in-situ observations. *Continental Shelf Research, 68*, 51–66.

Morrone, M.; Cappelletti, N.E.; Tatone, L.; Astoviza M.J.; Colombo, J.C. (2015). Passive sampling rates of dissolved contaminants in distilled and river water" M. Morrone, *SETAC Latin America 11th biennial meeting*. 7-10 de septiembre de 2015.

Morrone, M. (2017). Passive sampling of dissolved pollutants: Optimization of technique and calibration in laboratory and field at contrasting sites in the Plata basin. *PhD thesis National University of La Plata.*

Moy, F. & Walday, M. (1996). Accumulation and depuration of organic micro-pollutants in marine hard bottom organisms. *Marine Pollution Bulletin*, Vol. 33, Nos 1 6, pp. 56-63, 1996.

Namiesnik, J., Zabiegala, B., Kot-Wasik, A., Partyka, M., & Wasik, A. (2005). Passive sampling and/or extraction techniques in environmental analysis: a review. *Anal.Bioanal.Chem., 381*, 279–301.

Petersen, J., Paschke, A,. Gunold, R., Schüürmann, G. (2015). Calibration of Chemcatcher® passive sampler for selected highly hydrophobic organic substances under fresh and sea water conditions. *Environ. Sci.: Water Res. Technol.*, 2015, 1, 218.

Rodriguez-Ariza, A., Rodriguez-Ortega, M. J. J., Marenco, J. L. L., Amezcua, O., Alhama, J., & Lopez-Barea, J. (2003). Uptake and clearance of PCB congeners in Chamaelea gallina: Response of oxidative stress biomarkers. *Comparative Biochemistry and Physiology Part C, 134*(2003), 57–67.

Rusina, T. P., Smedes, F., Koblizkova, M., & Klanova, J. (2010). Calibration of silicone rubber passive samplers: Experimental and modeled relations between sampling rate and compound properties. *Environmental Science & Technology, 44*, 362–367.

Sericano, J.L., Wade, T.L., Jackson, T.J., Brooks, J.M., Tripp, B.W., Farrington, J.W., Mee, L.D., Readman, J.W., Villeneuve, J.-P., Goldberg, E.D., (1995). Trace organic contamination in the Americas: An overview of the US National status and trends and the international "Mussel Watch" programmes. *Marine Pollution Bulletin* 31, 214±225.

Shoults-Wilson, W.A., Unrine, J.M., Rickard, J., Black, M.C., (2010). Comparison of metal con- centrations in Corbicula fluminea and Elliptio hopetonensis in the Altamaha River system, Georgia, USA. *Environmental Toxicology and Chemistry* 29, 2026–2033.

U.S. EPA. Environmental Protection agency (2019). Final second five-year review report for the Hudson river PCBs superfund site. Appendix 1. Evelution of water column PCB concentrations and loadings. *Louis Berger US, Inc, LimnoTech*, Inc. April, 2019.

Tatone L., Skorupka C.N, Colombo J.C. (2020). Comprehensive evaluation of metal pollution and ecological risk in settling material from differently impacted sites in the Río de la Plata basin. *Human and Ecological Risk Assessment: An International Journal.*

Vrana, A. P., & Popp, P. (2006). Calibration and field performance of membrane-enclosed sorptive coating for integrative passive sampling of persistent organic pollutants in water. *Environmental Pollution, 144*(1), 296–307.

Vrana, B., Mills, G. A., & b Kotterman, M., Leonards, P., Booij, K., Greenwood, R. (2007). Modelling and field application of the Chemcatcher passive sampler calibration data for the monitoring of hydrophobic organic pollutants in wáter. *Environmental Pollution, 145*(2007), 895–904.

Vrana, B., Klucárová, V., Benická, E., Abou-Mrad, N., Amdany, R., Horáková, S., Draxler, A., Humer, F., & Gans, O. (2014). Passive sampling: An effective method for monitoring seasonal and spatial variability of dissolved hydrophobic organic contaminants and metals in the Danube river. *Environmental Pollution, 184*(2014), 101e112.

Webster, L., Russell, M., Phillips, L. A., Packer, G., Scurfield, J. A., Dalgarno, E. J., & Moffat, C. F. (2009). An assessment of persistent organic pollutants (POPs) in wild and rope grown blue mussels (Mytilus edulis) from Scottish coastal waters. *Journal of Environmental Monitoring, 11*, 1169–1184.

Yakushev, E., Pakhomova, S., Sørenson a, K., & Skei, J. (2009). Importance of the different manganese species in the formation of water column redox zones: Observations and modeling. *Marine Chemistry, 117*(2009), 59–70.