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Environmental Toxicology

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Metal concentrations in mussels from around the world

Trace Metal Residues in Marine Mussels: A Global Survey

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Abstract: Pressures from anthropogenic activities are causing degradation of estuarine and coastal ecosystems around the world. Trace metals are key pollutants that are released and can accumulate in a range of environmental compartments and are ultimately accumulated in exposed biota. The level of pressure varies with locations and the range and intensity of anthropogenic activities. This study measured residues of trace metals in *Mytilus* mussel species collected from a range of locations around the world in areas experiencing a gradient of anthropogenic pressures, that we classified as low, moderate or high impact. The data showed no grouping per impact level when sampling sites in all countries were incorporated in the analysis, but there was significant clustering per impact level for most countries. Overall, high impact areas were characterized by elevated concentrations of zinc, lead, nickel and arsenic, while copper and silver were detected in higher concentrations in medium impact areas. Finally, while most metals were in lower concentrations in areas classified as low impact, cadmium was typically elevated in these areas. This study provides a unique snapshot of worldwide levels of coastal metal contamination through the use of *Mytilus* species, a well-established marine biomonitoring tool.

Keywords: Marine mussels; Mytilus; Metals; International survey; Pollution; Bioaccumulation

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INTRODUCTION

Increasing agricultural, urban and industrial activities around coastal zones and the multiple stressors associated with these anthropogenic activities are producing profound ecological changes within estuaries and coastal zones (Jackson 2001; Paerl 2014). As a result, freshwater inputs into the marine environment contain a range of chemical contaminants of which trace metals are a major component (Eriksson 2007; Zgheib 2012). Assessing the extent of contamination by, and impacts of metals and other contaminants on ecosystem health is an ongoing priority for governments, scientists and managers worldwide (O'Brien 2016).

Mussels of the genus *Mytilus* have been well-characterized as bioindicators (sentinel species) as part of coastal biomonitoring programs (González-Fernández 2015) and are widely distributed in coastal areas throughout the world. They are sessile filter feeders and have a high propensity to accumulate both aqueous contaminants and those accumulated by the primary producers on which they feed (Beyer 2017). As a result, *Mytilus sp*. are used in a range of mussel watch programs in coastal countries throughout the world (Beyer 2017).

Several studies have used mussels as bioindicators of coastal health but to our knowledge, there has been no coordinated survey of metal residue concentrations in mussels collected in areas under different levels of anthropogenic contamination pressure around the world. The aim of this study was to compare metal residues in mussel soft tissues collected from sites varying in anthropogenic pressure, from seven countries across five continents. Collaborators used the same protocol to collect and sample mussels and metal analyses were conducted in the same laboratory, with the exception of the Spanish samples.

MATERIALS AND METHODS

Sampling sites

Mussels of the *Mytilus* genera were collected from Argentina, Canada, China, Mexico, New Zealand, South Africa and Spain from sites covering a gradient of anthropogenic pressure. Sampling was carried out in September 2016 in South Africa and between June and September 2017 elsewhere. The sites were assigned a low, medium, or high impact level based on the surrounding land-based activities (Table 1). These levels of impact were selected to provide some level of pollution gradient and were arbitrarily assigned. The levels were not supported by chemical data and caution is therefore warranted for the interpretation of the results, particularly among countries. For all sites except those in Spain, 10 mussels were collected from each site. Mussels were dissected and the tissues were dehydrated at 60˚C until constant weight, and shipped dry to the INRS-ETE laboratory for metal analysis, which was performed on individual mussels (10 per site). A different protocol was used for the samples from the Spain sites. Three pools of 50 individuals ranging between 35-50 mm were collected at each sampling site. In the laboratory, mussel tissues were separated from their shells and crushed using an Ultraturrax.

Trace metal analysis

All metal analyses except for the Spanish samples were performed at the INRS-ETE (Grasset 2016). Briefly, all laboratory material used for sample preparation and analysis was soaked in a nitric acid solution (HNO_3 -15%) for 24 h and then rinsed five times with distilled water and three times with ultrapure water to avoid metal contamination. Mussel soft tissues were freeze-dried and homogenized with an agate mortar. Subsamples were taken after the homogenization step for metal analysis and digested at room temperature in 100 µL of nitric acid (trace metal grade) per mg of dry weight sample. After three days, hydrogen peroxide was added at a ratio of 40 µL per mg of dry weight sample and left to rest for 24 h. Finally, ultrapure water was added to obtain a final concentration of 10% HNO_3. Concentrations for Ni, Cu, Zn, As, Ag, Cd and Pb were determined with an inductively coupled plasma–mass spectrometer (ICP–MS; Thermo Elemental X Series, Winsford, England, UK).

Throughout the freeze-drying and digestion steps, two certified standards (TORT-2 (lobster hepatopancreas) and DORT-4 (dogfish liver), National Research Council of Canada, NRCC, Halifax, NS, Canada) as well as blanks were processed along with the samples, to control for contamination and analytical accuracy. Mean recoveries (expressed as % of certified values ± SEM) of TORT-2 reference standard were Ni: 85.2 ± 4%; Cu: 98.7 ± 0.3%; Zn: 93.6 ± 4.2%; As: 96.2 ± 1.6%; Cd: 95 ± 3.1% and Pb: 84 ±

5%. For DORT-4, recovery rates were: Ni: 94.2 ± 4.2%; Cu: 100 ± 1.3%; Zn: 96.3 ± 2.1%; As: 87 ± 2%; Ag: 94 ± 5%; Cd: 95 ± 2% and Pb: 90.5 ± 2.5%.

Different methods were used to analyze the Spanish samples. The tissue samples were digested with nitric acid (Suprapur, Merck) in a microwave oven (Besada 2014). Briefly, 0.3 g of freeze-dried mussel samples were placed in a high-pressure Teflon reactor, and after the addition of nitric acid, digested in a microwave oven at 90°C for 10 min and then at 180°C for 60 min. A Perkin-Elmer A Analyst 800 spectrophotometer, equipped with a Zeeman background correction device (Cd, Pb and As by electrothermal AAS) was used throughout. Detection limits were 0.005, 0.050, 0.50, 0.30, 0.30 mg/kg dry weight (mg/kg dw) for Cd, Pb, Cu, Zn and As, respectively. Analysis of Ni and Ag was not performed on the Spanish samples. The analytical methods were subjected to a strict quality assurance and quality control (QA/QC). Protocols involved use of certified reference materials, duplicated samples and procedural blanks. In addition, the laboratory participates regularly in international interlaboratory exercises organized by QUASIMEME (Quality Assurance of Information in Marine Environmental Monitoring in Europe) Laboratory Performance Studies and IAEA (International Atomic Energy Agency), consistently demonstrating analytical accuracy. The analytical studies for trueness yielded results within the certified confidence intervals of the *Mytilus edulis,* CRM 278R (European Community Bureau of Reference, BCR) material. Experimental values were as follows: Cd: 0.353 ± 0.018; Pb 2.01 ± 0.03; Cu 9.48 ± 0.36; Zn 82.5 ± 2.9 and As 6.11 ± 0.25. In addition, recovery studies carried out by spiking aliquots of the reference material yielded average recoveries for the overall analytical methodology between 101.3% (Cu) and 98.3% (Cd).

Statistical analysis

Statistical analyses were carried out with the R software (R Core Team 2021). Metal concentration differences among locations and impact levels were visualised with boxplots using the 'ggpubr' R package (version 0.4.0) (Kassambara 2018), and with Principal Components Analysis (PCA) using the 'vegan' R package (version 2.5.6) (Oksanen 2008). Significant differences between samples of different impact levels were tested with Permutational analysis of variance (Permanova; 999 permutations) using the 'adonis' function of 'vegan' and pairwise permutational analysis using the 'pairwiseAdonis' R package (0.4.0) (Martinez Arbizu 2020) for Euclidean methods. In addition, a Partial Least Squares discriminant analysis (PLS-DA) and Receiver Operating Characteristic (ROC) curve, calculated from training cross-validation sets and averaged, were performed with the 'mixOmics' R package (version 6.10.9) (Rohart 2017). Prior to creating the PCA, PLS-DA and performing statistical analysis, metal concentrations were standardized using the 'scale' function from base R.

RESULTS AND DISCUSSION

The distribution and concentrations of individual metals at all locations are summarised in Table 2 with additional details provided in Figures S1 and S2.

They illustrate the wide spread of concentrations across sites and levels of impact. It is noteworthy that mussels sampled in Spain had by far the highest concentration of zinc, regardless of the impact level (Figure S1). Spanish Zn levels were similar to concentrations reported for mussels from the Tagus estuary (Portugal) (260–484 µg/g dw; (Santos 2014), the Atlantic French coast (36-406 µg/g dw (Ifremer 2006)) or from the

Moroccan Atlantic coast (107–366 µg/g dw (Maanan 2007)). Furthermore, the Spanish data are in agreement with already published data by the researchers for sampling sites of the same area (Besada 2011; Besada 2014) or in other sites in the Spanish Cantabrian coast (203–374 µg/g dw (Bartolomé 2010)) or in the Mediterranean coast (91-431 µg/g dw (Fernández 2012)).

Figure 1 shows that metal concentration profiles weakly clustered per location. Mussels from China were associated with high concentrations of Ni, Cd and Cu, in contrast to mussels from the New Zealand, Quebec and South Africa sites, which clustered together in an area of the plot representing lower concentrations of these metals. Mussels from the Argentina, British Columbia and Mexico sites globally clustered together in the centre of the plot, suggesting intermediate levels of metal concentrations. No evident grouping per impact level could be observed in the whole dataset when data from all locations were pooled. Looking at each location individually however, we can observe significant (Figure 1, Table 3) clustering per impact level for most sites, with a very clear grouping for Spain.

Overall, relatively high stochasticity in metal concentrations was observed among impact levels (Figures S1-S2-S3). Maximising the covariance among samples of different impact categories resulted in a better grouping of the samples (Figure 2A), and better predicted samples belonging to high impact environments (AUC = 0.9; Figure 2B). Predictions for the low and medium impact levels were more difficult as these samples tended to overlap more (AUC = 0.6 and 0.66, respectively; Figure 2A-B). Based on the PSL-DA analysis, metals that contributed most to the high impact level were, in decreasing order, zinc, lead, nickel and arsenic (Figure 2C-D). The medium impact samples were mostly characterized by higher concentrations of copper and silver while cadmium tended to be higher in low impact samples (Figure 2C-D). In support of this conclusion, the Argentinian site, classified as Low Impact, has been monitored for several years by the “Program for monitoring the environmental quality of production areas in the province of Rio Negro”, reporting natural cadmium as the only metal present at high levels (Sturla Lompré 2020).

Although it is well established that bivalves can accumulate metals from the water column (Beyer 2017), tissue metal residues may not accurately reflect water contamination levels. Metal bioaccumulation data should be considered in combination with metal concentrations measured in all ecosystem compartments (Zuykov 2013). Nevertheless, mussels have been successfully used to assess spatial distribution and temporal trends of trace metals as part of the Mussel Watch program (Santos-Echeandía 2021).

The current study provides a snapshot overview of metal residues in *Mytilus* species around the world. Such study is challenging as it requires the coordination of multiple research groups across a range of regions and jurisdictions. We were successful in coordinating the collection and sampling of mussel using one agreed protocol. The aim was focussed on metals, but a similar approach can be used for organic contaminant residues. As it is only a one-off sample, some caution with the interpretation of the data is warranted. There are many confounding factors including the species, age distribution and reproductive stage. It was demonstrated that *Mytilus* spp. show differences in chemical bioaccumulation and it is important to identify the species for environmental monitoring studies (Brooks 2015).In addition, metal burden in mussels is seasonally influenced (Knopf 2020) and samples from the Northern and Southern hemispheres were simultaneously collected to achieve the snapshot, resulting in opposite seasons.

Supporting Information—The Supporting information are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Data availability—Data, associated metadata, and calculation tools are available from the corresponding author (patrice.couture@inrs.ca).

REFERENCES

Bartolomé L, Navarro P, Raposo JC, Arana G, Zuloaga O, Etxebarria N, Soto M. 2010. Occurrence and distribution of metals in mussels from the cantabrian coast. *Arch Environ Contam Toxicol* 59:235-243. DOI: 10.1007/s00244-010-9476-7.

Besada V, Andrade JM, Schultze F, Gonzalez JJ. 2011. Monitoring of heavy metals in wild mussels (*Mytilus galloprovincialis*) from the Spanish North-Atlantic coast. *Cont Shelf Res* 31:457-465. DOI: 10.1016/j.csr.2010.04.011.

Besada V, Sericano JL, Schultze F. 2014. An assessment of two decades of trace metals monitoring in wild mussels from the Northwest Atlantic and Cantabrian coastal areas of Spain, 1991-2011. *Environ Int* 71:1-12. DOI: 10.1016/j.envint.2014.05.024.

Beyer J, Green NW, Brooks S, Allan IJ, Ruus A, Gomes T, Bråte ILN, Schøyen M. 2017. Blue mussels (*Mytilus edulis* spp.) as sentinel organisms in coastal pollution monitoring: A review. *Mar Environ Res* 130:338-365. DOI: 10.1016/j.marenvres.2017.07.024.

Brooks SJ, Farmen E, Heier LS, Blanco-Rayón E, Izagirre U. 2015. Differences in copper bioaccumulation and biological responses in three *Mytilus* species. *Aquat Toxicol* 160:1-12. DOI: 10.1016/j.aquatox.2014.12.018.

Eriksson E, Baun A, Scholes L, Ledin A, Ahlman S, Revitt M, Noutsopoulos C, Mikkelsen PS. 2007. Selected stormwater priority pollutants - a European perspective. *Sci Total Environ* 383:41-51. DOI: 10.1016/j.scitotenv.2007.05.028.

Fernández B, Campillo JA, Martínez-Gómez C, Benedicto J. 2012. Assessment of the mechanisms of detoxification of chemical compounds and antioxidant enzymes in the digestive gland of mussels, *Mytilus galloprovincialis*, from Mediterranean coastal sites. *Chemosphere* 87:1235-1245. DOI: 10.1016/j.chemosphere.2012.01.024.

González-Fernández C, Albentosa M, Campillo JA, Viñas L, Fumega J, Franco A, Besada V, González-Quijano A, Bellas J. 2015. Influence of mussel biological variability on pollution biomarkers. *Environ Res* 137:14-31. DOI: 10.1016/j.envres.2014.11.015.

Grasset J, Ollivier É, Bougas B, Yannic G, Campbell PGC, Bernatchez L, Couture P. 2016. Combined effects of temperature changes and metal contamination at different levels of biological organization in yellow perch. *Aquat Toxicol* 177:324-332. DOI: 10.1016/j.aquatox.2016.06.008.

Ifremer. 2006. RNO. Surveillance du Milieu Marin. Travaux du Réseau National d'Observation de la qualité du milieu marin. Edition 2006.

Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, Hughes TP, Kidwell S, Lange CB, Lenihan HS, Pandolfi JM, Peterson CH, Steneck RS, Tegner MJ, Warner RR. 2001. Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293:629-638. DOI: 10.1126/science.1059199.

Kassambara A. 2018. ggpubr: “ggplot2” Based Publication Ready Plots. R package version 0.4.0.

Knopf B, Fliedner A, Radermacher G, Rüdel H, Paulus M, Pirntke U, Koschorreck J. 2020. Seasonal variability in metal and metalloid burdens of mussels: using data from the German Environmental Specimen Bank to evaluate implications for long-term mussel monitoring programs. *Environ Sci Eur* 32. DOI: 10.1186/s12302-020-0289-7.

Maanan M. 2007. Biomonitoring of heavy metals using *Mytilus galloprovincialis* in Safi coastal waters, Morocco. *Environ Toxicol* 22:525-531. DOI: 10.1002/tox.20301.

Martinez Arbizu P. 2020. pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 0.4.0.

O'Brien A, Townsend K, Hale R, Sharley D, Pettigrove V. 2016. How is ecosystem health defined and measured? A critical review of freshwater and estuarine studies. *Ecol Indicators* 69:722-729. DOI: 10.1016/j.ecolind.2016.05.004.

Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2008. vegan: Community Ecology Package (R package version 1.15-1).

Paerl HW, Hall NS, Peierls BL, Rossignol KL. 2014. Evolving Paradigms and Challenges in Estuarine and Coastal Eutrophication Dynamics in a Culturally and Climatically Stressed World. *Estuaries Coast* 37:243-258. DOI: 10.1007/s12237-014-9773-x.

R Core Team. 2021. R: A language and environment for statistical computing. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rohart F, Gautier B, Singh A, Lê Cao KA. 2017. mixOmics: An R package for ‘omics feature selection and multiple data integration. *PLoS Comp Biol* 13. DOI: 10.1371/journal.pcbi.1005752.

Santos-Echeandía J, Campillo JA, Egea JA, Guitart C, González CJ, Martínez-Gómez C, León VM, Rodríguez-Puente C, Benedicto J. 2021. The influence of natural vs anthropogenic factors on trace metal(loid) levels in the Mussel Watch programme: Two decades of monitoring in the Spanish Mediterranean sea. *Mar Environ Res* 169:105382. DOI: 10.1016/j.marenvres.2021.105382.

Santos I, Diniz MS, Carvalho ML, Santos JP. 2014. Assessment of essential elements and heavy metals content on *Mytilus galloprovincialis* from river Tagus estuary. *Biol Trace Elem Res* 159:233-240. DOI: 10.1007/s12011-014-9974-y.

Sturla Lompré J, Malanga G, Gil MN, Giarratano E. 2020. Multiple-biomarker approach in a commercial marine scallop from San Jose gulf (Patagonia, Argentina) for health status assessment. *Arch Environ Contam Toxicol* 78:451-462. DOI: 10.1007/s00244-019-00690-1.

Zgheib S, Moilleron R, Chebbo G. 2012. Priority pollutants in urban stormwater: Part 1-Case of separate storm sewers. *Water Res* 46:6683-6692. DOI: 10.1016/j.watres.2011.12.012.

Zuykov M, Pelletier E, Harper DAT. 2013. Bivalve mollusks in metal pollution studies: From bioaccumulation to biomonitoring. *Chemosphere* 93:201-208. DOI: 10.1016/j.chemosphere.2013.05.001.

Figures

Figure 1. A) Principal components analysis with samples coloured per sampling region and shaped per impact level. Metal concentration gradients are overlaid as arrows. Samples from Spain were excluded as they did not have data for Ni and Ag. B) Principal components analysis per location, with samples coloured per impact level. Metal concentration gradients are overlaid as arrows. The Argentina, China and Quebec samples were omitted as they only had data for one impact level.

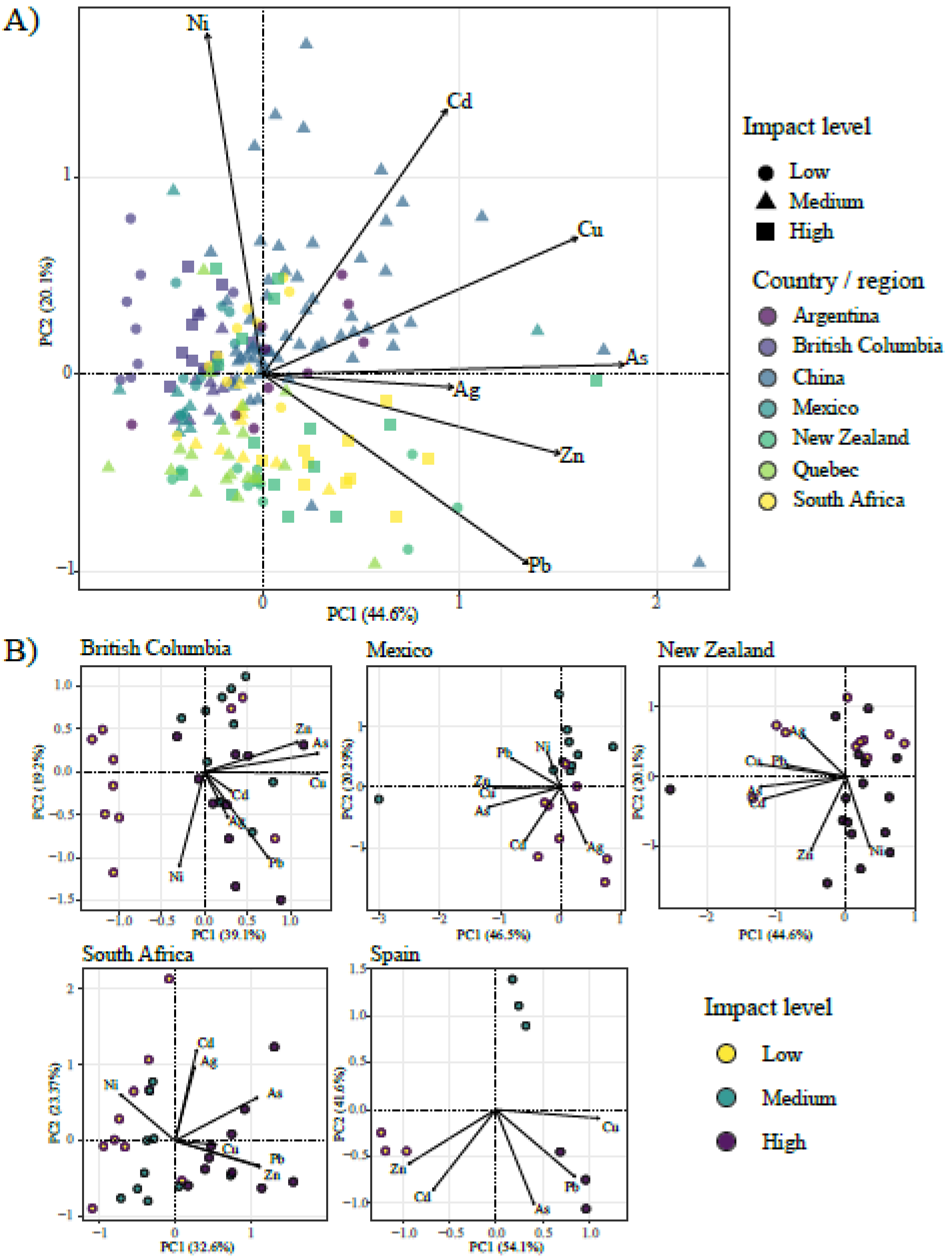
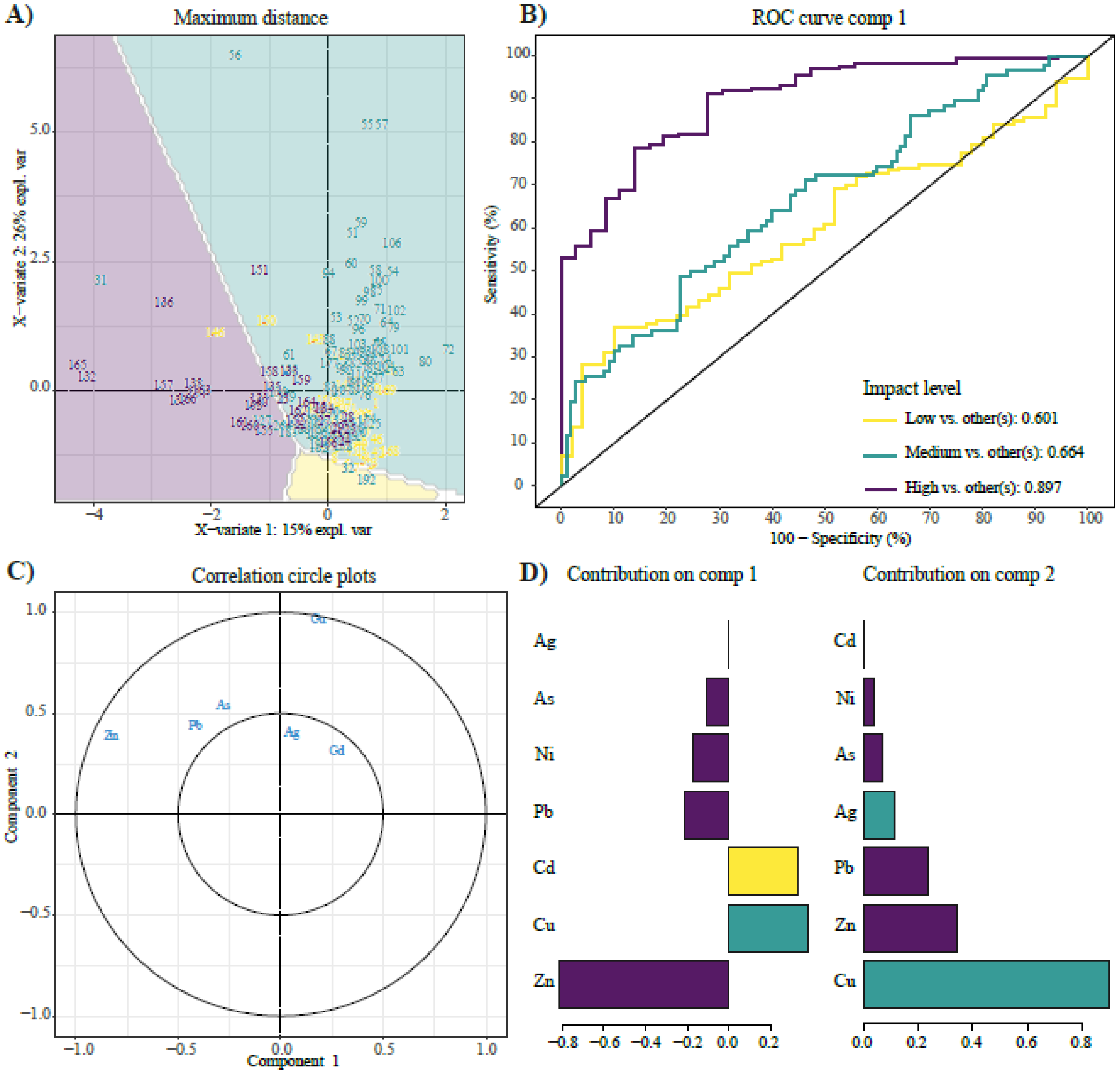


Figure 2. Classification A) Partial Least Squares discriminant analysis (PLS-DA) with a prediction background based on Rohart et al. (2017), B) Receiver Operating Characteristic (ROC) curve calculated from training cross-validation sets and averaged, C) correlation circle plot indicating ordination of the concentration gradient per metal, D) contribution of each variable (metal) per component, coloured by the impact level to which it contributes the most. Samples from Spain were excluded as no data were available for nickel and silver.



Tables

Table 1. Description of the sites where mussels from the *Mytilus* genera were collected.

Country	Site	Coordinates	Site description	Impact level
Argentina	PO	40.9000 S 64.5166 W	Playa Orengo sector, approximately 37 km east of the mouth of San Antonio Bay	Low
Canada	WP	49.3731 N 123.2886 W	Whytecliff Park, BC. Popular local park and scuba destination with little boat traffic	Low
	SM	49.1710 N 123.1410 W	Sunset Marina, BC. Local marina serving Lions Bay impacted by local boat traffic	Medium
	SP	49.2931 N 123.1230 W	Stanley Park, BC. Active shipping harbour and prime cruise ship destination. One of Canada's most popular tourist attractions	High
	RM	48.4806 N 68.5098 W	Rimouski marina, Quebec. Small local fishing port, servicing the local (mostly) sailing community	Medium
	IM	47.4405 N 61.7744 W	Îles-de-la-Madeleine, Quebec. The Havre-aux-Maisons marina services the local boating community through the summer season	Medium
China	FZ	26.0528 N 119.7053 E	Fuzhou, a coastal city, the estuary of the Minjiang River, East China Sea	Medium

	DA	39.0081 N 121.0742 E	Dalian Bay, a famous tourist city, the junction of the Bohai Sea and the Yellow Sea	Medium
Mexico	CM	31.8616 N 116.6650 W	Coral y Marina. Marina servicing a hotel in a sheltered harbour	Medium
	BR	31.7192 N 116.6684 W	Rincon de las Ballenas open coast. Certified area for shellfish aquaculture	Low
New Zealand	PB	41.051469 S 173.465968 E	Penzance Bay, in the Marlborough Sounds New Zealand, receives minimal anthropogenic input	Low
	PN	41.153549 S 173.161591 E	Port of Nelson, New Zealand, large commercial shipping port	High
South Africa	SC	34.1989 S 18.3884 E	Scarborough, a small rural settlement near Cape Point and adjacent to a National Park	Low
	BL	33.7992 S 18.4560 E	Bloubergstrand, a peripheral low-density suburb of Cape Town about 10 km from the city centre and with no industrial input	Medium
	GP	33.9120 S 18.4088 E	Green Point, a high-rise suburb close to centre of Cape Town close to a large sewage discharge	High
Spain	OI	41.9702 N 8.8866 W	Oia open coast, clean site. Intense upwelling	Low
	RO	43.3824 N	Mera, mouth of the Coruña Ria, urban area and harbour activities	Medium

		8.3439 W		
	AV	43.5793 N 5.9696 W	Aviles open coast. Industrial, urban and mining activities, and a harbour	High

Table 2. Mean metal concentration (μg/g dw) and standard deviation (within brackets) per country, site and impact level

Country	Site	Impact level	Ag	As	Cd	Cu	Ni	Pb	Zn
Argentina	PO	Low	0.32 (0.33)	20.55 (8.44)	7.81 (4.08)	9.89 (2.73)	0.64 (0.77)	1 (0.41)	13.3 (5.54)
Canada	WP	Low	0.12 (0.04)	4.59 (4.41)	4.01 (1.83)	5.48 (4.93)	2.53 (1.27)	0.48 (0.23)	6.13 (6.35)
	SM	Medium	0.17 (0.12)	10.93 (1.79)	4.09 (1.23)	9.11 (1.36)	1.11 (0.67)	0.6 (0.19)	10.88 (3.7)
	SP	High	0.14 (0.05)	9.34 (1.85)	3.07 (0.89)	10.63 (2.39)	2.68 (0.92)	0.89 (0.36)	14.2 (8.1)
	IM	Medium	0.32 (0.37)	14.65 (6)	0.65 (0.3)	6.65 (2.75)	0.29 (0.39)	1.54 (0.67)	13.16 (4.55)
	RM	Medium	0.32 (0.71)	12.29 (4.82)	2.26 (1.09)	9.23 (5.42)	1.29 (1.6)	2.47 (2.27)	15.73 (5.97)
China	DA	Medium	0.17 (0.16)	14.07 (4.68)	7.28 (3.55)	25.25 (8.16)	1.76 (1.25)	1.27 (0.36)	19.32 (5.11)
	FZ	Medium	0.84 (1.73)	14.85 (5.86)	6.36 (4.18)	33.94 (15.55)	1.59 (1.14)	2.12 (3.91)	18.4 (9.6)
Mexico	BR	Low	0.28 (0.27)	11.42 (3.74)	5.49 (1.59)	7.8 (6.83)	1.26 (0.84)	0.41 (0.34)	7.68 (3.17)
	CM	Medium	0.1 (0.07)	11.2 (10.91)	2.41 (2)	10.32 (7.72)	1.76 (1.84)	0.8 (0.37)	19.04 (27.32)
New Zealand	PB	Low	0.47 (0.39)	19.14 (11.73)	1.54 (1.68)	13.8 (6.57)	0.12 (0)	3.32 (2.29)	16.37 (11.94)
	PN	High	0.54 (0.52)	16.53 (12.25)	1.81 (1.99)	11.68 (6.64)	1.92 (2.09)	2.18 (0.85)	36.73 (19.48)
South Africa	SC	Low	0.19 (0.25)	10.02 (3.31)	7.68 (3.35)	9.9 (4.68)	1.37 (0.45)	1.38 (1.05)	19.34 (3.92)
	BL	Medium	0.21 (0.26)	8.6 (1.45)	7.04 (2.33)	5.45 (1.4)	0.12 (0)	2.31 (1.96)	24.04 (8.69)
	GP	High	0.28 (0.22)	15.51 (2.49)	6.7 (2.41)	10.26 (5.99)	0.12 (0)	3.39 (0.62)	51.15 (23.22)
Spain	OI	Low		14.83 (0.55)	2.17 (0.13)	5.8 (0.24)		1.14 (0.42)	424.33 (10.79)

RO	Medium	13.87 (0.55)	0.63 (0.05)	7.05 (0.13)		1.77 (0.32)	309 (10.58)
AL	High	16 (0.56)	1.53 (0.16)	7.84 (0.35)		28.17 (3.48)	333.33 (19.35)

Table 3. Permutational analysis of variance (Permanova) between impact level samples per region, including pair-wise comparisons. The Argentina, China and Quebec samples were omitted as they only had data for one impact level.

	Permanova		Pairwise Permanova		
Region	R^2	p value	Pair	R^2	Adj. p value
British Columbia	0.27	**0.001**	Low vs Medium	0.22	**0.012**
			Low vs High	0.24	**0.018**
			Medium vs High	0.19	**0.006**
Mexico	0.15	**0.003**	Low vs Medium	0.15	**0.001**
New Zealand	0.10	**0.029**	Low vs High	0.10	**0.029**
South Africa	0.36	**0.001**	Low vs Medium	0.24	**0.003**
			Low vs High	0.35	**0.003**
			Medium vs High	0.29	**0.003**
Spain	0.93	**0.007**	Low vs Medium	0.92	0.300

	Low vs High	0.90	0.300
	Medium vs High	0.89	0.300