

Spatial and seasonal assessment of heavy metal contamination in the sea urchin *Paracentrotus lividus* and the mussel *Mytilus galloprovincialis* from three ports of the Algerian west coast

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ABSTRACT

This study assessed spatial and seasonal variation in cadmium, lead, copper, zinc and mercury in the sea urchin *Paracentrotus lividus* and the mussel *Mytilus galloprovincialis* at three harbour stations on the Algerian west coast. Monthly sampling from January to December 2025 at one fixed station in each of Oran, Arzew and Mostaganem yielded 216 composite samples, accompanied by biometric and environmental measurements. Metals were quantified by atomic absorption spectrometry following microwave digestion. Spatial comparisons used station–month means with month as a blocking factor, supported by mixed models, robust tests and adjustment for body size. Inference was restricted to the three sampled stations because independent spatial replication within ports was absent. Both species exhibited the same concentration ranking, Oran > Arzew > Mostaganem, for all five metals, with significant pairwise differences after Tukey adjustment. Station and season effects were significant, without a significant interaction; concentrations peaked in summer at approximately 1.35 times winter levels. The station effect persisted after adjustment for body size. Mussels accumulated more cadmium, lead, zinc and mercury, whereas copper concentrations were higher in sea urchins. Allometric exponents were 2.774 for sea urchins and 2.932 for mussels. After adjustment for species and season and correction for multiple testing, metal concentrations remained associated with turbidity and suspended particulate matter at Oran and with temperature and dissolved oxygen at Mostaganem; no association persisted at Arzew. These relationships were interpreted as associations rather than evidence of contaminant pathways. The metal pollution index reproduced the spatial ranking, decreasing from 2.77 at Oran to 1.65 at Mostaganem. The findings document persistent differences among the sampled stations and complementary accumulation patterns in the two species, supporting their combined use in regional biomonitoring. Generalisation to entire ports requires additional independent stations.

Keywords: heavy metals, biomonitoring, *Paracentrotus lividus*, *Mytilus galloprovincialis*, atomic absorption spectrometry, biometry, Algerian coast.

INTRODUCTION

Coastal waters adjoining large ports receive a sustained input of trace metals from commercial shipping, dredging, urban runoff, untreated

domestic discharge and industrial effluent, and semi-enclosed basins such as the Mediterranean retain these inputs for long periods because water renewal is slow (Belabed et al., 2017; Castrec et al., 2023). Trace metals are neither degraded nor

readily eliminated, so they accumulate in sediments and in the tissues of benthic organisms, where they may reach concentrations several orders of magnitude above those of the surrounding water (Yehdih et al., 2022). Along the Algerian coast, where population density and petrochemical activity are concentrated in a narrow coastal strip, documenting the magnitude and spatial structure of this contamination remains an operational priority for environmental management (Kaddour et al., 2022; Haddidi et al., 2026).

Chemical analysis of the water column alone is a poor descriptor of biologically available contamination, because dissolved metal concentrations fluctuate over hours to days and represent only a small part of the total metal pool. Sessile or low-mobility invertebrates integrate exposure over weeks to months and therefore give a more stable and ecologically meaningful signal, which is why they are the basis of most regional monitoring networks (Barre et al., 2024). The Mediterranean mussel *Mytilus galloprovincialis* is the most widely applied of these sentinels: it is abundant, easy to sample, tolerant of contamination, and its filter-feeding habit exposes it continuously to both the dissolved fraction and suspended particles (Kapranov et al., 2023; Martinović et al., 2023). Its use in trace metal surveys is well established across the western Mediterranean and adjacent Atlantic coasts (Azizi et al., 2021a; Peycheva et al., 2023).

Echinoids offer a complementary perspective. The purple sea urchin *Paracentrotus lividus* is a grazing herbivore that lives in close contact with rocky substrates and associated sediment, so its exposure route differs fundamentally from that of a suspension feeder, and it has been proposed as a sentinel of coastal water quality in its own right (Garcês and Pires, 2023; González-Delgado et al., 2024). Trace element burdens in *P. lividus* respond to local contamination gradients and vary seasonally with the reproductive and feeding cycle (El Idrissi et al., 2020; El Idrissi et al., 2023). The species is also of direct economic relevance in Algeria, where its gonads are consumed, and recent national surveys have documented substantial metal burdens in its tissues (Yahiaoui Bouiba et al., 2024).

Deploying both a suspension feeder and a grazer at the same sites strengthens inference considerably. When two species with different feeding modes, tissue compositions and metal-handling physiologies rank a set of sites in the

same order, the resulting gradient is unlikely to be an artefact of the biology of either one, and divergences between them become informative about the pathway by which a given element enters the food web (Klaoudatos et al., 2022; El Boudam-moussi et al., 2024). Earlier Algerian work has examined the two taxa largely in isolation, with separate studies on mussels (Guendouzi et al., 2018; Boudjema et al., 2022). Urchins have likewise been surveyed on their own along the same coast (Rouane-Hacene et al., 2018). Comparative multi-site datasets that combine metal burdens, biometric condition and concurrent physico-chemical measurements therefore remain scarce for the western part of the coast.

The present study was designed to fill that gap. Over a complete annual cycle, both species were sampled monthly at one station in each of three ports of the Algerian west coast that span a gradient of urban and industrial pressure, namely Oran, Arzew and Mostaganem. The objectives were to quantify cadmium, lead, copper, zinc and mercury in soft tissues of both species; to partition the variation in metal burdens between site and season and test whether the spatial pattern is stable through the year; to compare the two species as accumulators of each element; to describe biometric condition and length–weight relationships at each port; and to relate tissue burdens to locally measured physico-chemical conditions. The contribution is primarily empirical: a year-round regional dataset for two functionally different bioindicators sampled at the same stations with concurrent water measurements, and a comparative assessment of the two species as accumulators.

MATERIALS AND METHODS

Study area and sampling design

Sampling was conducted at three harbours on the Algerian west coast, distributed over approximately 90 km of shoreline in the Gulf of Oran and the adjacent coastal sector. The Port of Oran is a major commercial and passenger harbour bordered by a dense urban area of more than one million inhabitants and receives substantial untreated urban runoff. The Port of Arzew serves one of the largest petrochemical and liquefied natural gas complexes in the southern Mediterranean, and is therefore dominated by industrial rather than

urban inputs. The Port of Mostaganem is a smaller mixed fishing and commercial harbour with markedly lower shipping traffic and no heavy industry in its immediate catchment; it was included as the least impacted reference site of the three.

The geographical position of the three sampling stations is shown in Figure 1. Their coordinates (WGS 84) are 35.7135° N, 0.6556° W for the Oran station, 35.8616° N, 0.2961° W for the Arzew station and 35.9313° N, 0.0731° E for the Mostaganem station (Table S1). The three harbours therefore lie on the same coastal stretch, share the same regional climate and the same prevailing west-to-east longshore circulation, and differ essentially in the nature and intensity of the pressures exerted on them. Within each port a single fixed station was sampled throughout the survey, located on the seaward face of the breakwater closest to the harbour and reached from the adjacent shore rocks, because access to the enclosed harbour areas is prohibited; the three ports are therefore represented by three stations and the inferential unit of the spatial comparison is the station rather than the port as a whole (coordinates, depth and substrate in Table S1).

At each station, both species were collected monthly for twelve consecutive months (January to December 2025) from the same shallow subtidal rocky station, at depths of 1–3 m. Campaigns took place on the Sunday or Monday of the first week of each month (07:30–11:00, calm to slightly rough sea, light west-north-westerly wind); one team visited the three stations in turn, except from March to June when three teams

sampled them simultaneously under the same protocol (Table S1). Sea urchins were collected by hand in the infralittoral zone (1–3 m) and mussels were scraped from the hard substrate of the intertidal fringe of the same station, along a fixed stretch of shore, taking every adult encountered within the retained size window until the required number was reached; individuals were then allocated at random to the three composite samples. On each sampling occasion, three replicate composite samples were prepared per species, each pooling 20–30 adult individuals of comparable size, giving a total of 5,460 individuals across the survey (2,709 *P. lividus* and 2,751 *M. galloprovincialis*); the exact number of individuals in every composite sample is given in Table S2 and in the supplementary dataset, and was identical for the three replicates of a given station and month. The resulting design comprised 3 stations $\times$ 12 months $\times$ 3 replicates $\times$ 2 species, that is 216 composite samples; the three replicates of a station and month are subsamples of the same station and not independent spatial replicates (see Statistical analysis). Animals were transported to the laboratory in insulated containers with seawater from the sampling site and processed on the day of collection. Only apparently healthy adults were retained; damaged or visibly parasitised individuals were discarded in the field. The stations lie outside the restricted harbour areas and were reached from the public shore; no permit is required in Algeria for the collection of these non-protected invertebrates in the quantities taken, and no ethical approval applies to these species.



Figure 1. Location of the three sampling ports along the Algerian west coast. The inset shows the position of the study area in the southwestern Mediterranean basin

Biometric measurements

For *P. lividus*, test diameter was measured at the ambitus with a digital caliper to the nearest 0.01 mm, and total wet weight was recorded to the nearest 0.01 g on an analytical balance. Gonads were dissected and weighed, and the gonadosomatic index was calculated as:

$$GSI = 100 W_G / W \quad (1)$$

where: W_G is the gonad wet weight, W the total body wet weight (g).

For *M. galloprovincialis*, maximum shell length was measured along the antero-posterior axis to the nearest 0.01 mm and total wet weight was recorded to the nearest 0.01 g. A Fulton-type condition index was computed for each species as:

$$K = 100 W / L^3 \quad (2)$$

where: W is the total wet weight (g), L the test diameter or the shell length (cm), following the general approach used in Mediterranean mussel and urchin monitoring (Grković et al., 2020; Abbassi et al., 2022).

All biometric values reported here are means of the individuals pooled within each composite replicate; individual measurements were not archived separately, so the composite mean is the unit of all biometric analyses.

Heavy metal analysis

All analytical work was carried out jointly in the Laboratory of Toxicology, Environment and Health (LATES) of the University of Science and Technology of Oran Mohamed Boudiaf and in the Laboratory Network for Environmental Monitoring (LRSE) of the University of Oran 1 Ahmed Ben Bella.

Reagents and labware

All chemicals were of trace-metal grade: nitric acid (HNO_3 , 67–69%, Suprapur, Merck), hydrogen peroxide (H_2O_2 , 30%, Suprapur, Merck) and ultrapure water (Milli-Q, resistivity $\geq 18.2 \text{ M}\Omega \text{ cm}$). Polypropylene tubes were soaked overnight in 10% HNO_3 and rinsed three times with ultrapure water before use. Working calibration standards were freshly prepared by dilution of 1,000 mg L^{-1} single- and multi-element stock solutions (Inorganic Ventures) over element-specific ranges matched to the expected sample concentrations, namely 0–5

$\mu\text{g L}^{-1}$ for mercury and cadmium, 0–20 $\mu\text{g L}^{-1}$ for lead, 0–5 mg L^{-1} for copper and 0–2 mg L^{-1} for zinc, and matrix-matched to the digestion medium.

Sample preparation and microwave digestion

Soft tissues were removed with acid-washed stainless steel instruments, rinsed with ultrapure water, blotted and weighed fresh to the nearest 0.1 mg, then freeze-dried at -55°C for 48 h (Christ Alpha 1-2 LD plus), reweighed and homogenised with an agate mortar to a fine powder. About 0.25 g of each dry homogenate was weighed into perfluoroalkoxy digestion vessels and treated with 7 mL HNO_3 and 1 mL H_2O_2 . Digestion was performed in a closed-vessel microwave system (Anton Paar Multiwave PRO) following a programmed cycle of a 10 min ramp to 180°C , a 15 min hold at 180°C and 35 bar, and a 20 min cooling step. Digests were quantitatively transferred to 50 mL polypropylene tubes and brought to volume with ultrapure water.

Multi-element determination by atomic absorption spectrometry

Cadmium, lead, copper and zinc were determined on a GBC Avanta 932 atomic absorption spectrometer fitted with element-specific hollow-cathode lamps. Copper and zinc, present at milligram per litre level in the digests, were measured by flame atomic absorption spectrometry in an air–acetylene flame at 324.7 and 213.9 nm respectively, whereas cadmium and lead, present at microgram per litre level, were measured by graphite-furnace atomic absorption spectrometry at 228.8 and 217.0 nm (Table 1). Calibration was performed for each element against the matrix-matched aqueous standards described above, over a working range bracketing the expected sample concentrations; all calibration curves were linear with a coefficient of determination of at least 0.999, and the calibration was verified periodically during each analytical run.

Mercury determination by cold-vapour atomic absorption spectrometry

Total mercury was determined by cold-vapour atomic absorption spectrometry on a dedicated flow-injection mercury system (Perkin Elmer

FIMS-100) coupled to the same spectrometer, with stannous chloride (SnCl_2 , 1.1% w/v in 3% HCl) as the reducing agent. Detection was carried out at 253.7 nm in a quartz absorption cell.

Quality assurance and quality control

Accuracy and precision were verified with two certified reference materials of marine invertebrate soft tissue: ERM-CE278k mussel tissue, certified by the Joint Research Centre of the European Commission, and IAEA-452 scallop soft tissue, certified by the International Atomic Energy Agency. Both are certified for the five elements determined here and their certified mass fractions bracket the ranges measured in the samples. No certified reference material of echinoderm origin is commercially available, so the same two materials were used for both species, as is current practice in trace element work on *P. lividus*. One aliquot of reference material was digested and analysed alongside every batch of twenty samples, under the same conditions as the samples. Recoveries ranged from 92 to 108% for the five elements. Procedural blanks ($n = 12$) were systematically below the limit of detection, and triplicate analyses of each sample gave a relative standard deviation below 8%. Limits of detection were derived from three times the standard deviation of the procedural blanks and limits of quantification from the conventional relationship in which the limit of quantification equals ten thirds of the limit of detection, both expressed on a dry-weight basis (Table 1). Reagent blanks were carried through the complete digestion and measurement procedure and subtracted from the sample readings. Every concentration reported here exceeded the corresponding limit of quantification by at least a factor of four, so no value had to be

treated as censored. Concentrations are expressed in mg kg^{-1} dry weight. The certified values and identification of the two reference materials, the recovery acceptance range met in every batch, the instrumental repeatability, the calibration scheme and the batch acceptance criteria are compiled in Table S3 of the Supplementary Online Material; the analytical quality statement signed by the laboratory is supplied as a separate file. No spike-recovery test on the urchin matrix was performed; the absence of a matrix-specific bias for *P. lividus* is therefore inferred from the bivalve reference materials only, which is acknowledged as a limitation.

The dataset comprises 1,080 individual metal determinations (216 composite samples $\times$ 5 elements). Four copper values, corresponding to 0.37% of determinations, could not be validated and were treated as missing; they were excluded from the corresponding analyses rather than imputed, and the reduced sample sizes are indicated in the relevant tables.

Physico-chemical monitoring

At every sampling event, water temperature, salinity, dissolved oxygen, pH, turbidity and suspended particulate matter were measured in situ at the same station and time as the biological collection, in three replicates. Temperature and pH were recorded with a multi-parameter probe, dissolved oxygen with an optical oxygen probe, salinity with a hand-held refractometer and turbidity with a portable nephelometer, all calibrated before each campaign (Table S1); suspended particulate matter was determined gravimetrically by filtering a measured volume of seawater through pre-dried, pre-weighed $0.45 \mu\text{m}$ membrane filters dried at 105°C to constant weight. Water replicate

Table 1. Atomisation mode, analytical wavelength and performance of the atomic absorption spectrometry determinations

Element	Technique	Wavelength (nm)	LOD (mg kg^{-1} dw)	LOQ (mg kg^{-1} dw)
Mercury (Hg)	CV-AAS	253.7	0.002	0.007
Cadmium (Cd)	GF-AAS	228.8	0.001	0.003
Lead (Pb)	GF-AAS	217.0	0.005	0.017
Copper (Cu)	F-AAS	324.7	0.010	0.033
Zinc (Zn)	F-AAS	213.9	0.050	0.167

Note: CV-AAS – cold-vapour atomic absorption spectrometry; GF-AAS – graphite-furnace atomic absorption spectrometry; F-AAS – flame atomic absorption spectrometry; LOD – limit of detection, taken as three times the standard deviation of twelve procedural blanks; LOQ – limit of quantification, taken as ten thirds of the limit of detection; dw – dry weight.

k ($k = 1$ to 3) was taken at the point of the station where composite sample k of each species was collected, so that every composite sample is paired with one water measurement of the same station, date and location; the paired observations are provided in the supplementary dataset. Pairing the environmental and biological measurements in space and time allows tissue burdens to be interpreted against the local hydrological context.

Statistical analysis

Analyses were carried out in Python 3 using the SciPy and statsmodels libraries; the complete code and the dataset are provided as Supplementary Online Material. Because each port was represented by one station, the three composite samples of a given station and month are subsamples of the same station rather than independent spatial replicates, and consecutive months are repeated observations of the same station. To avoid inflating the effective sample size, the spatial comparison was based on station–month means: for each species, the three composites were averaged per station and month, giving 36 units per species (3 stations $\times$ 12 months). The station effect was tested on these means by analysis of variance with station as a fixed factor and month as a blocking factor ($df = 2, 22$), which removes the temporal component shared by the three stations in a given month. The same data were also fitted with a linear mixed model with station and season as fixed effects and month as a random intercept, and the lag-1 autocorrelation of the station residuals was inspected. Differences among stations were then resolved with Tukey's honestly significant difference test applied to the station–month means separately for each species. As a descriptive summary of the variance structure at the composite level, for each metal and each species a two-way analysis of variance was fitted with site and season as fixed factors and their interaction, the seasons being defined as winter (December to February), spring (March to May), summer (June to August) and autumn (September to November). Normality of the model residuals was checked with the Shapiro–Wilk test and homogeneity of variance among the twelve site $\times$ season cells with Levene's test; where homogeneity was rejected, the station effect was retested with Welch's heteroscedastic analysis of variance, the Kruskal–Wallis test and analysis of variance of $\log_{10}$ -transformed concentrations. The

share of variance attributable to each term was expressed as partial eta squared (η^2_p), the ratio of the effect sum of squares to the sum of the effect and residual sums of squares; this quantity is relative to the effect and its error term within the fitted model and is not a share of the total observed variance. Because body size differed among stations, the robustness of the station effect to size was tested by analysis of covariance, refitting each model with site and season as factors and the mean test diameter or shell length of the composite sample as a covariate, after testing the homogeneity of regression slopes through the station $\times$ size interaction. Species were compared with a linear mixed model fitted to the station–month means with species, station and their interaction as fixed effects, season as a fixed effect and month as a random intercept, complemented by a paired t-test on the 36 station–month pairs; Welch's two-sample t-test on the pooled composites and Cohen's d are retained only as descriptive summaries of the overall contrast. One-way analysis of variance was used to compare physico-chemical and biometric variables among stations. Length–weight relationships were characterised both by the Pearson correlation on untransformed data and by linear regression of $\log_{10}$ -transformed values, the slope of which estimates the allometric exponent b; departure from isometry was tested against $b = 3$ with a t-test on the regression slope. Associations between tissue metal concentrations and physico-chemical variables were quantified with Pearson correlation coefficients computed separately within each station, so that the resulting coefficients reflect temporal covariation at a given port rather than the among-site contamination gradient. Each composite sample was paired with the water replicate collected at the same point and time ($n = 72$ paired observations per station, both species). Because temperature and dissolved oxygen have a marked seasonal component and the two species differ in mean concentration, the coefficients were also computed as partial correlations after removing the species and season effects from both variables by linear regression, and the 30 tests per station were corrected for multiple comparisons with the Benjamini–Hochberg false-discovery-rate procedure. To summarise the joint burden of the five elements in a single descriptor, a metal pollution index (MPI) was computed for every composite sample as the geometric mean of the five concentrations:

$$MPI = (C_1 \times C_2 \times \dots \times C_n)^{(1/n)} \quad (3)$$

where: C_i is the concentration of element i in $\text{mg} \cdot \text{kg}^{-1}$ dry weight.

This index is widely used to compare overall contamination among sites monitored with the same sentinel species (El Idrissi et al., 2023; El Mourabit et al., 2024). It is a dimensionless relative score that discards the identity of the individual elements, and it is used here only to rank stations and seasons within this study. Index values were compared among stations and seasons with the same two-way analysis of variance and Tukey procedure as the individual elements, and the station effect was confirmed on station–month means with month as block. Statistical significance was set at $p < 0.05$ throughout.

RESULTS

Physico-chemical characterisation of the three stations

The three stations differed sharply in water clarity and oxygenation, while differing only slightly

in salinity and pH. Mean values and among-site comparisons are given in Table 2, and the distributions of the three most discriminating variables are shown in Figure 2. Turbidity and suspended particulate matter decreased steeply from Oran through Arzew to Mostaganem, with an approximately threefold difference between the extremes, and dissolved oxygen followed the reverse order. Temperature varied over a typical annual range of 13.41–26.85 °C and did not differ among sites, and salinity (36.24–37.54 psu) and pH (7.98–8.26) varied within narrow ranges across the study area. The three stations therefore constitute a gradient of particulate load and organic enrichment rather than a gradient of basic hydrological character.

Spatial variation in tissue metal concentrations

Tissue burdens of all five metals differed significantly among the three stations in both species, and the ranking was identical in every case, namely Oran > Arzew > Mostaganem (Table 3, Figure 3). Pooling species, mean cadmium declined from $0.669 \pm 0.204 \text{ mg kg}^{-1}$ at Oran to

Table 2. Physico-chemical characteristics of the water column at the three stations (mean $\pm$ SD, $n = 36$ per site) and one-way analysis of variance among sites

Parameter	Oran	Arzew	Mostaganem	F	p
Temperature (°C)	19.90 $\pm$ 4.33	19.45 $\pm$ 4.24	18.94 $\pm$ 4.22	0.45	0.638
Salinity (psu)	37.19 $\pm$ 0.17	37.09 $\pm$ 0.17	36.80 $\pm$ 0.26	35.27	<0.001
Dissolved oxygen (mg L^{-1})	6.17 $\pm$ 0.27	6.57 $\pm$ 0.33	6.98 $\pm$ 0.30	65.17	<0.001
pH	8.07 $\pm$ 0.06	8.10 $\pm$ 0.04	8.15 $\pm$ 0.05	22.06	<0.001
Turbidity (NTU)	12.64 $\pm$ 1.93	7.25 $\pm$ 1.24	3.83 $\pm$ 1.07	332.73	<0.001
SPM (mg L^{-1})	32.50 $\pm$ 4.52	19.29 $\pm$ 3.28	9.38 $\pm$ 2.57	383.99	<0.001

Note: SPM – suspended particulate matter. F and p refer to the one-way analysis of variance testing differences among the three stations ($df = 2, 105$).

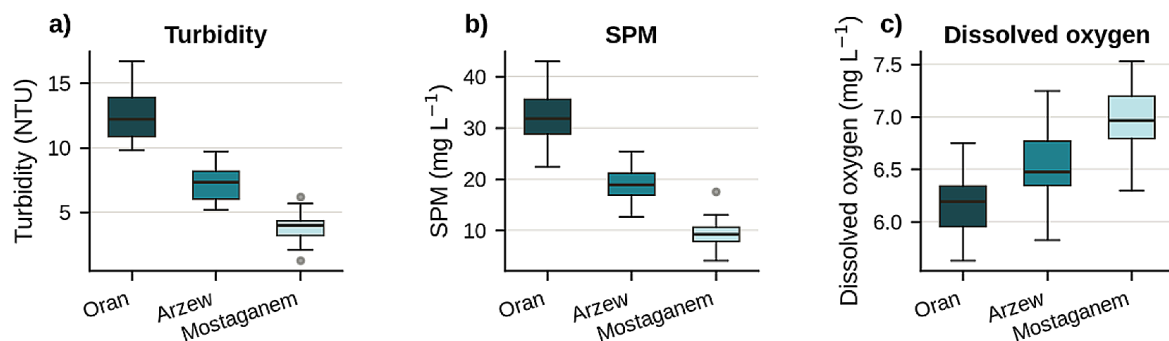


Figure 2. Distribution of the three most discriminating physico-chemical variables at the three stations over the annual cycle: (a) turbidity, (b) suspended particulate matter, (c) dissolved oxygen. Boxes span the interquartile range, the horizontal line is the median, whiskers extend to 1.5 times the interquartile range and points are values beyond that limit ($n = 36$ per site)

$0.402 \pm 0.111 \text{ mg kg}^{-1}$ at Mostaganem, and lead from 2.255 ± 0.667 to $1.333 \pm 0.362 \text{ mg kg}^{-1}$. Zinc was by far the most abundant element, falling from 115.786 ± 56.434 to $68.708 \pm 30.779 \text{ mg kg}^{-1}$, whereas mercury was the least abundant, declining from 0.098 ± 0.031 to $0.058 \pm 0.017 \text{ mg kg}^{-1}$. The site effect was highly significant for every metal in both species, with F values between 83 and 130 (Table 3); these composite-level statistics are descriptive, since the three composites of a station and month are subsamples of the same station. When the analysis was based on

station–month means with month as a blocking factor, the station effect remained highly significant for every metal in both species ($F(2,22) = 75\text{--}158$, $p < 0.001$; Table 4), and the linear mixed model with month as a random intercept led to the same conclusion (Wald $\chi^2 = 151\text{--}316$, $p < 0.001$; Table S4). The lag-1 autocorrelation of the station residuals ranged from -0.41 to 0.45 with a mean close to zero, giving no indication of serial dependence beyond the seasonal term. Partial eta squared of the composite-level model, expressed relative to each term plus the residual variance,

Table 3. Tissue concentrations of the five metals (mg kg^{-1} dry weight, mean $\pm$ SD) at each station for each species, and descriptive two-way analysis of variance at the composite level with site and season as fixed factors

Metal	Species	Oran	Arzew	Mostaganem	F site	F season	F site $\times$ season
Cd	<i>P. lividus</i>	0.512 ± 0.094	0.419 ± 0.062	0.319 ± 0.068	102.66	26.37	1.57
Cd	<i>M. galloprovincialis</i>	0.826 ± 0.156	0.665 ± 0.113	0.486 ± 0.079	106.44	17.67	1.09
Pb	<i>P. lividus</i>	1.740 ± 0.329	1.400 ± 0.229	1.060 ± 0.193	101.82	21.93	1.21
Pb	<i>M. galloprovincialis</i>	2.769 ± 0.499	2.172 ± 0.394	1.606 ± 0.275	117.21	19.33	1.28
Cu	<i>P. lividus</i>	12.224 ± 2.100	10.205 ± 1.794	7.594 ± 1.464	90.74	21.94	0.91
Cu	<i>M. galloprovincialis</i>	9.522 ± 1.722	7.528 ± 1.308	5.403 ± 0.917	126.19	19.06	1.06
Zn	<i>P. lividus</i>	64.526 ± 11.448	52.934 ± 9.586	40.526 ± 6.750	94.69	22.27	1.47
Zn	<i>M. galloprovincialis</i>	167.046 ± 30.401	132.119 ± 23.366	96.891 ± 15.566	114.47	17.66	0.99
Hg	<i>P. lividus</i>	0.073 ± 0.014	0.059 ± 0.010	0.045 ± 0.010	83.01	18.51	1.18
Hg	<i>M. galloprovincialis</i>	0.124 ± 0.022	0.097 ± 0.016	0.070 ± 0.013	129.49	19.48	0.92

Note: $n = 36$ per site and species, except copper in *Paracentrotus lividus* ($n = 34$ at Oran, $n = 35$ at Arzew and Mostaganem). All site and season effects were significant at $p < 0.001$; no site $\times$ season interaction was significant ($p > 0.16$ in all cases); the inferential test of the station effect is given in Table 4.

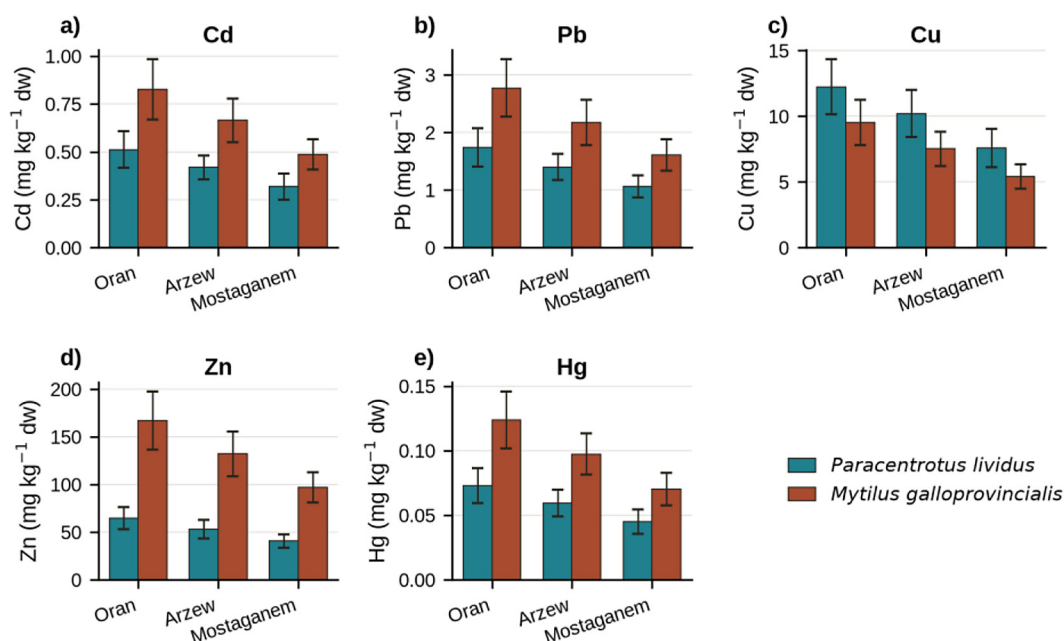


Figure 3. Mean tissue concentrations of (a) cadmium, (b) lead, (c) copper, (d) zinc and (e) mercury in *Paracentrotus lividus* and *Mytilus galloprovincialis* at the three stations. Bars are annual means and error bars are standard deviations ($n = 36$ per species and site, except copper in *P. lividus*)

Table 4. Station effect on tissue metal concentrations tested on station–month means (analysis of variance with month as a blocking factor, $n = 36$ per species) and pairwise differences between stations (mg kg^{-1} dry weight) with Tukey adjusted probabilities, for each species

Metal	Species	F(2,22)	p	Oran – Arzew	p	Oran – Mostaganem	p	Arzew – Mostaganem	p
Cd	<i>P. lividus</i>	90.9	<0.001	−0.093	0.004	0.194	<0.001	0.101	0.002
Pb	<i>P. lividus</i>	98.3	<0.001	−0.34	0.002	0.68	<0.001	0.34	0.002
Cu	<i>P. lividus</i>	137.7	<0.001	−1.90	0.011	4.56	<0.001	2.66	<0.001
Zn	<i>P. lividus</i>	119.5	<0.001	−11.59	0.003	24.00	<0.001	12.41	0.001
Hg	<i>P. lividus</i>	75.3	<0.001	−0.014	0.003	0.028	<0.001	0.014	0.002
Cd	<i>M. galloprovincialis</i>	136.9	<0.001	−0.161	<0.001	0.340	<0.001	0.179	<0.001
Pb	<i>M. galloprovincialis</i>	152.7	<0.001	−0.60	<0.001	1.16	<0.001	0.57	<0.001
Cu	<i>M. galloprovincialis</i>	139.6	<0.001	−1.99	<0.001	4.12	<0.001	2.13	<0.001
Zn	<i>M. galloprovincialis</i>	157.4	<0.001	−34.93	<0.001	70.16	<0.001	35.23	<0.001
Hg	<i>M. galloprovincialis</i>	158.0	<0.001	−0.026	<0.001	0.053	<0.001	0.027	<0.001

Note: Each station–month mean is the average of the three composite samples of that station and month. Positive differences indicate a higher concentration at the first station of each pair. All contrasts were significant at $p < 0.05$.

was 0.63–0.73 for site, 0.36–0.45 for season and at most 0.09 for the interaction (Table 7); these values describe the variance structure of that model and are not shares of the total variance. Model residuals did not depart from normality in any case (Shapiro–Wilk $p = 0.09$ –0.98), and variances were homogeneous across the twelve site $\times$ season cells for eight of the ten metal–species combinations (Levene $p \geq 0.08$); for the two exceptions, lead in the urchin ($p = 0.038$) and mercury in the mussel ($p = 0.014$), as for all other combinations, the station effect was confirmed by Welch’s heteroscedastic analysis of variance ($F = 53$ –91), by the Kruskal–Wallis test ($H = 57$ –71) and by analysis of variance of $\log_{10}$ -transformed concentrations ($F = 82$ –145), all at $p < 0.001$, and after transformation the variances were homogeneous in every case (Levene $p \geq 0.20$; Table S4).

Tukey’s test applied to the station–month means of each species confirmed that all three stations were significantly different from one another for every metal in both species (adjusted $p \leq 0.011$; Table 4), so the gradient is not driven by a single divergent station. The largest contrast was consistently between Oran and Mostaganem, and the Oran–Arzew and Arzew–Mostaganem contrasts were of comparable magnitude, indicating a graded rather than a stepwise pattern.

Seasonal variation

Season contributed significantly to the variance of every metal in both species, but always far less than site, and the site $\times$ season interaction

was never significant (Table 3); the mixed model on station–month means likewise retained a significant season effect (Wald $\chi^2 = 17$ –23, $p < 0.001$; Table S4). The spatial ranking of the three stations was therefore preserved in every season. Concentrations followed a summer maximum and a winter minimum: pooling species and sites, lead rose from 1.548 mg kg^{-1} in winter to 2.092 mg kg^{-1} in summer, a ratio of 1.35, and copper from 7.530 to 10.256 mg kg^{-1} . Monthly means for lead illustrate this pattern and show that the three sites remained clearly separated throughout the year (Figure 4a). The seasonal amplitude was modest relative to the among-site range: the summer maximum at Mostaganem remained below the winter minimum at Oran for lead, cadmium and mercury. Distributions by season and species are shown in Figure 4b.

Interspecific differences in metal accumulation

The two species differed significantly for all five elements, but not in the same direction (Table 5). *M. galloprovincialis* contained substantially more cadmium, lead, zinc and mercury than *P. lividus*; the contrast was largest for zinc, at 2.51 times the urchin concentration, and amounted to 1.58-fold for cadmium, 1.56-fold for lead and 1.64-fold for mercury. Copper reversed this pattern and was higher in *P. lividus* ($9.986 \pm 2.607 \text{ mg kg}^{-1}$) than in the mussel ($7.484 \pm 2.159 \text{ mg kg}^{-1}$). Effect sizes were large for every element, and all differences were significant at $p < 0.001$. In the

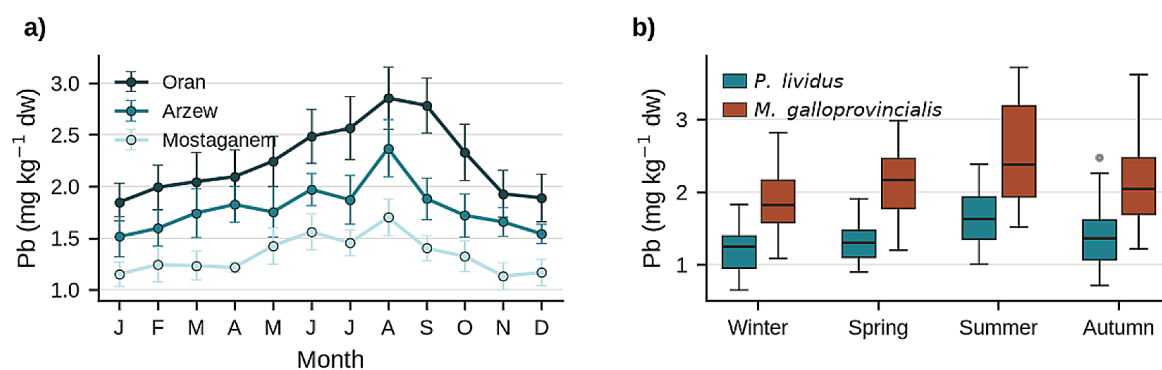


Figure 4. Seasonal dynamics of lead: (a) monthly means at the three stations, both species pooled, with standard errors; (b) distribution by season and species. Months are indicated by their initial letter from January to December

mixed model fitted to the station–month means, which accounts for station, season and month, the species effect was significant for all five metals (Wald $\chi^2 = 100\text{--}471$, $p < 0.001$), as was the paired comparison of the 36 station–month means ($|t| = 17\text{--}21$, $p < 0.001$; Table 5). The species $\times$ station interaction was significant for cadmium, lead, zinc and mercury ($p < 0.001$) but not for copper ($p = 0.33$): the excess of the mussel over the urchin widened from Mostaganem to Oran for the four elements, whereas the copper reversal was of similar magnitude at the three stations. The two species therefore do not simply duplicate one another as monitoring tools.

Biometric characteristics and allometry

Length–weight relationships were tight and highly significant in both species (Figure 5a, 5b). In *P. lividus*, test diameter and total wet weight were correlated at $r = 0.971$, and regression of

$\log_{10}$ -transformed values gave an allometric exponent $b = 2.774 \pm 0.044$ ($R^2 = 0.974$), significantly below the isometric value of 3 ($t = -5.14$, $p < 0.001$). In *M. galloprovincialis*, shell length and total wet weight were correlated at $r = 0.969$, with $b = 2.932 \pm 0.048$ ($R^2 = 0.972$), which did not differ significantly from isometry ($t = -1.41$, $p = 0.162$). Growth was therefore close to isometric in the mussel and moderately negatively allometric in the urchin.

Body size differed markedly among stations in both species (Table 6, Figure 5c). Individuals from Mostaganem were the largest, with a mean test diameter of 48.51 mm in the urchin against 38.40 mm at Oran, and a mean shell length of 57.39 mm in the mussel against 45.35 mm at Oran. Gonadal investment in the urchin followed the same order, the gonadosomatic index at Mostaganem being about one third higher than at the two more impacted stations. The condition index of the mussel did not differ significantly among sites, and that of the urchin differed only marginally.

Table 5. Comparison of mean metal concentrations (mg kg⁻¹ dry weight) between the two species over the whole survey: descriptive Welch t-test and Cohen's d on pooled composites, and inferential linear mixed model on station–month means with species, station, their interaction and season as fixed effects and month as a random intercept, with the paired t-test on the 36 station–month pairs

Metal	<i>P. lividus</i>	<i>M. galloprovincialis</i>	Ratio M/P	Welch t	Cohen's d	χ^2 sp.	χ^2 sp. $\times$ st.	p sp. $\times$ st.	Paired t
Cd	0.417 $\pm$ 0.109	0.659 $\pm$ 0.184	1.58	-11.78	1.60	175.4	31.4	<0.001	17.4
Pb	1.400 $\pm$ 0.378	2.182 $\pm$ 0.620	1.56	-11.20	1.52	164.5	32.2	<0.001	17.7
Cu	9.986 $\pm$ 2.607	7.484 $\pm$ 2.159	0.75	7.60	-1.05	100.5	2.2	0.328	-18.3
Zn	52.662 $\pm$ 13.593	132.019 $\pm$ 37.257	2.51	-20.79	2.83	470.9	80.0	<0.001	20.6
Hg	0.059 $\pm$ 0.016	0.097 $\pm$ 0.028	1.64	-12.26	1.67	193.8	43.5	<0.001	17.0

Note: $n = 108$ per species, except copper in *Paracentrotus lividus* ($n = 104$). The ratio is the mussel mean divided by the urchin mean; values below one indicate higher concentrations in the urchin. A negative Welch t value and a positive paired t value indicate a higher mean in the mussel; all Welch, mixed-model and paired tests were significant at $p < 0.001$. χ^2 sp. and χ^2 sp. $\times$ st. – Wald statistics of the mixed model for the species effect ($df = 1$) and the species $\times$ station interaction ($df = 2$), with the probability of the interaction; the paired t-test has 35 degrees of freedom.

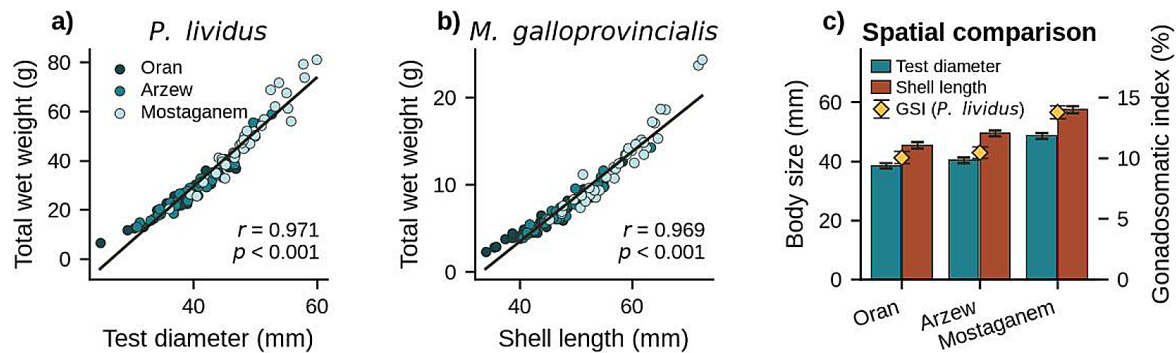


Figure 5. Biometric relationships and spatial comparison: (a) test diameter against total wet weight in *Paracentrotus lividus*; (b) shell length against total wet weight in *Mytilus galloprovincialis*; (c) mean body size of both species and gonadosomatic index of the urchin at the three stations. In (a) and (b) the solid line is the least-squares fit and point colours denote the sampling port; in (c) error bars are standard errors

Because body size differed among stations, the site effect on each metal was re-examined with the mean size of the composite sample as a covariate (Table 7). Adjustment for size reduced the F statistic of the site effect by about half on

average but left it highly significant for all five metals in both species ($F = 45\text{--}59$, $p < 0.001$). The covariate itself was not significant for any metal in the urchin, and was significant only for cadmium, zinc and mercury in the mussel, with

Table 6. Biometric descriptors of both species at the three stations (mean $\pm$ SD, $n = 36$ per species and site) with one-way analysis of variance among sites

Species	Parameter	Oran	Arzew	Mostaganem	F	p
<i>P. lividus</i>	Test diameter (mm)	38.40 $\pm$ 5.03	40.29 $\pm$ 5.50	48.51 $\pm$ 5.36	37.04	<0.001
<i>P. lividus</i>	Total wet weight (g)	25.53 $\pm$ 8.50	29.72 $\pm$ 11.84	48.93 $\pm$ 14.72	39.17	<0.001
<i>P. lividus</i>	Gonado-somatic index (%)	10.05 $\pm$ 2.96	10.46 $\pm$ 2.92	13.81 $\pm$ 3.12	16.96	<0.001
<i>P. lividus</i>	Condition index	43.60 $\pm$ 3.60	43.59 $\pm$ 3.43	41.80 $\pm$ 3.27	3.29	0.041
<i>M. galloprovincialis</i>	Shell length (mm)	45.35 $\pm$ 6.51	49.39 $\pm$ 5.46	57.39 $\pm$ 6.72	34.57	<0.001
<i>M. galloprovincialis</i>	Total wet weight (g)	6.34 $\pm$ 2.75	8.07 $\pm$ 2.62	12.49 $\pm$ 4.45	31.77	<0.001
<i>M. galloprovincialis</i>	Condition index	6.44 $\pm$ 0.55	6.49 $\pm$ 0.49	6.37 $\pm$ 0.51	0.51	0.604

Note: F and p refer to the one-way analysis of variance among the three stations ($df = 2, 105$).

Table 7. Partial eta squared of the site, season and interaction terms of the two-way analysis of variance, and site effect on tissue metal concentrations before and after adjustment for body size by analysis of covariance, with site and season as factors and the mean test diameter (urchin) or shell length (mussel) of each composite sample as covariate

Metal	Species	η^2p site	η^2p season	η^2p site $\times$ season	F site, unadjusted	F site, size-adjusted	p site, adjusted	F size	p size
Cd	<i>P. lividus</i>	0.68	0.45	0.09	102.66	52.79	<0.001	1.34	0.250
Pb	<i>P. lividus</i>	0.68	0.41	0.07	101.82	55.53	<0.001	0.79	0.377
Cu	<i>P. lividus</i>	0.66	0.42	0.06	90.74	49.01	<0.001	0.52	0.471
Zn	<i>P. lividus</i>	0.66	0.41	0.08	94.69	48.64	<0.001	1.43	0.235
Hg	<i>P. lividus</i>	0.63	0.37	0.07	83.01	46.94	<0.001	0.17	0.679
Cd	<i>M. galloprovincialis</i>	0.69	0.36	0.06	106.44	45.21	<0.001	6.69	0.011
Pb	<i>M. galloprovincialis</i>	0.71	0.38	0.07	117.21	53.50	<0.001	3.82	0.053
Cu	<i>M. galloprovincialis</i>	0.72	0.37	0.06	126.19	58.50	<0.001	3.53	0.063
Zn	<i>M. galloprovincialis</i>	0.70	0.36	0.06	114.47	51.09	<0.001	5.48	0.021
Hg	<i>M. galloprovincialis</i>	0.73	0.38	0.05	129.49	58.80	<0.001	5.76	0.018

Note: η^2p – partial eta squared. Unadjusted F values are those of the two-way analysis of variance (Table 3). Adjusted values come from the model with site, season and size; $n = 108$ per species ($n = 104$ for copper in *Paracentrotus lividus*).

negative slopes indicating slightly lower concentrations in larger animals. The homogeneity of regression slopes assumed by the analysis of covariance was supported for nine of the ten combinations (station $\times$ size interaction $p = 0.07$ – 0.99); the exception was lead in the mussel ($p = 0.034$), for which the size-adjusted station effect should be read with this caveat (Table S5). The adjustment addresses the size difference among stations but does not add spatial replication.

Metal burdens and local environmental conditions

Each composite sample was paired with the water measurement taken at the same point and time, giving 72 paired observations per station (36 per species). Raw Pearson correlations within each station showed a positive association of tissue concentrations with temperature, turbidity and suspended particulate matter and a negative one with dissolved oxygen (Table S6: 18, 14 and 9 of the 30 coefficients significant after false-discovery-rate correction at Oran, Arzew and Mostaganem respectively). Because temperature and dissolved oxygen carry a strong seasonal signal and the two species differ in mean concentration, the coefficients were recomputed as partial correlations after removing the species and season effects, with the same correction (Figure 6). At Oran, 16 associations remained significant: all five metals with temperature ($r = 0.49$ – 0.61), turbidity (0.34 – 0.44) and suspended particulate

matter (0.43 – 0.50), and mercury with dissolved oxygen (-0.28). At Mostaganem, 11 associations persisted, with temperature (0.36 – 0.47) and dissolved oxygen (-0.54 to -0.36) for all five metals and with turbidity for mercury only. At Arzew none of the 30 partial correlations remained significant ($|r| \leq 0.24$). Salinity and pH were unrelated to metal burdens at every station. The coupling with particulate load is therefore specific to the Oran station, whereas the temperature and oxygen associations persisting at Mostaganem cannot be separated from residual seasonal covariation and are not interpreted as evidence of a metal input pathway.

Integrated metal pollution index

The metal pollution index condenses the five elements into a single value per sample and reproduces the spatial ranking obtained element by element (Table 8, Figure 7). Pooling both species, the index decreased from 2.77 ± 0.82 at Oran to 2.23 ± 0.59 at Arzew and 1.65 ± 0.42 at Mostaganem, all three stations differing significantly from one another after Tukey adjustment ($p < 0.001$ for the three contrasts), with a difference of 1.12 units between the two extremes. On station–month means with month as block, the station effect on the index was $F(2,22) = 64.5$ in *P. lividus* and 159.9 in *M. galloprovincialis* ($p < 0.001$), with all Tukey contrasts significant (adjusted $p \leq 0.010$).

Within each species the site effect was again the dominant term, with $F = 87.5$ in *P. lividus* and

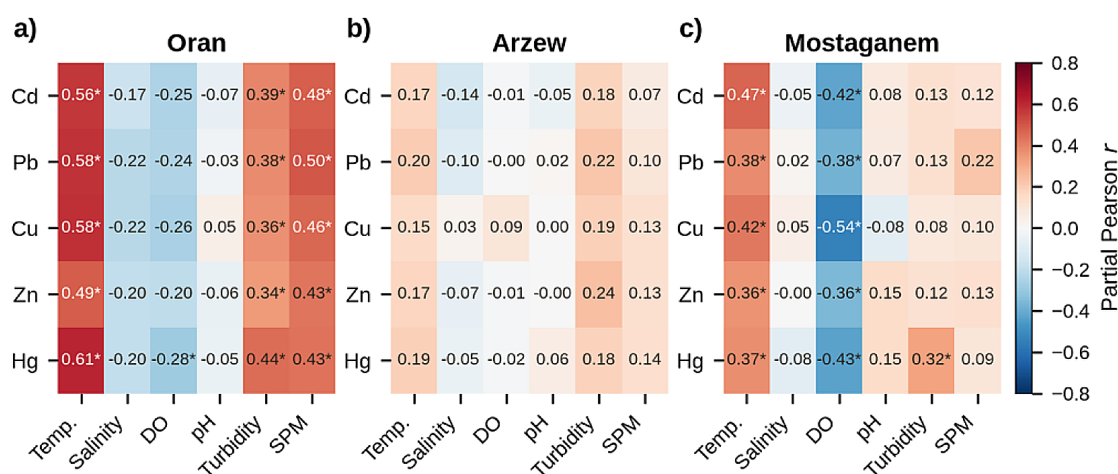


Figure 6. Partial Pearson correlation coefficients between tissue metal concentrations and physico-chemical variables, after removal of the species and season effects, computed separately within (a) Oran, (b) Arzew and (c) Mostaganem, both species pooled ($n = 72$ paired observations per station). Asterisks mark coefficients significant at a false-discovery rate of 5% within each station (30 tests). DO – dissolved oxygen; SPM – suspended particulate matter

Table 8. Metal pollution index of the two sentinel species at the three stations and two-way analysis of variance of the index

Species	Oran	Arzew	Mostaganem	F site	F season	F site × season
<i>P. lividus</i>	2.156 ± 0.441	1.777 ± 0.296	1.345 ± 0.261	87.50	25.00	1.67
<i>M. galloprovincialis</i>	3.392 ± 0.611	2.683 ± 0.455	1.954 ± 0.312	127.64	20.02	1.08

Note: MPI – metal pollution index, geometric mean of the cadmium, lead, copper, zinc and mercury concentrations expressed in mg kg⁻¹ dry weight; values are means ± standard deviation of n = 36 composite samples per site and species (n = 35, 35 and 34 for *Paracentrotus lividus* at Arzew, Mostaganem and Oran respectively). The site and season effects were significant at p < 0.001 in both species; the interaction was not significant (p > 0.13).

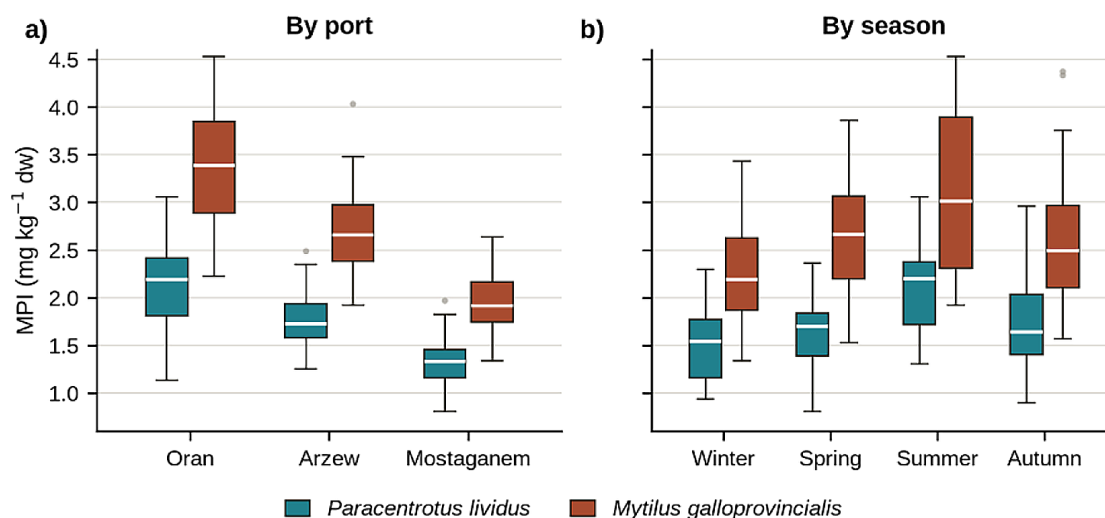


Figure 7. Metal pollution index of *Paracentrotus lividus* and *Mytilus galloprovincialis* (a) at the three stations and (b) across the four seasons. Boxes show the median and interquartile range, whiskers the 1.5 interquartile range and points the values beyond it

F = 127.6 in *M. galloprovincialis* (p < 0.001), while the season effect was significant but smaller (F = 25.0 and F = 20.0, p < 0.001) and the site × season interaction remained non-significant in both species (p = 0.136 and p = 0.378). Pooled seasonal means rose from 1.92 in winter to 2.60 in summer, a ratio of 1.36 that matches the amplitude observed for the individual elements. The index was 1.52 times higher in the mussel (2.68 ± 0.76) than in the urchin (1.76 ± 0.47; t = -10.69, p < 0.001), a difference driven mainly by zinc, the most abundant of the five elements.

DISCUSSION

A stable spatial contamination gradient

The most robust outcome of this survey is the reproducibility of the spatial ranking among the three harbour stations. All five metals, in two species with unrelated feeding modes, ordered the

three stations identically, every pairwise contrast was significant in each species after correction for multiple testing, and the ordering held in each of the four seasons and under every alternative analysis applied: station-month means with month as block, mixed models with month as a random effect, heteroscedasticity-robust and rank-based tests, logarithmic transformation and adjustment for body size. The absence of a site × season interaction is important in practical terms: it means that a survey conducted in any single season would have recovered the same ranking, which is a useful property for monitoring programmes that cannot sustain monthly sampling.

The ordering is consistent with the pressures exerted on each port, although this reading is associative. Oran combines intense commercial and passenger traffic with the runoff of a large conurbation, and showed both the highest metal burdens and the highest turbidity and particulate load. Arzew is dominated by petrochemical and gas activity and occupied an intermediate

position, while Mostaganem, with limited traffic and no heavy industry, was the least contaminated on every measure. Comparable associations between urban and industrial pressure and trace metal burdens have been reported along the eastern Algerian coast and its watersheds (Belabed et al., 2017) and in small Mediterranean harbours elsewhere (Castrec et al., 2023). Site-dependent burdens governed by harbour and urban pressure have likewise been described in mussels from the Algerian coast (Guendouzi et al., 2018). The sources of the metals were not measured directly in this study: no sediment, dissolved or particulate metal concentrations, discharge data or source-tracing analyses are available, so the attribution of the gradient to urban and industrial inputs is a plausible interpretation consistent with the observations rather than an established mechanism.

Table 9 places the present concentrations alongside recent surveys of the same two species in the Mediterranean and adjacent basins, restricted to studies reporting the analytical basis explicitly, and grouped by tissue and weight basis. Only the dry-weight, whole-soft-tissue series for the mussel are directly comparable with

the present data; the gonad and wet-weight series are listed for reference and are not compared quantitatively. On a dry-weight basis, cadmium and zinc in mussels from the three Algerian stations were of the same order as those recorded on the Moroccan Mediterranean coast (Azizi et al., 2021a; El Boudammoussi et al., 2024). Lead and copper were higher: the mean lead concentration in mussels from Oran (2.77 mg kg^{-1} dry weight) was more than four times the upper value reported for Moroccan mussels. For *P. lividus*, no recent dry-weight series on whole soft tissue was found; the gonad values of Sarly et al. (2023) and Mamede et al. (2022) refer to a different tissue and the wet-weight mussel series from the Marmara and Black Seas (Hacısalıhoğlu, 2025; Peycheva et al., 2023) cannot be converted without moisture data, so no position of the Algerian stations relative to these surveys is claimed. The ordering agrees with the earlier identification of Oran harbour as the most contaminated of three western Algerian urchin sites (Rouane-Hacene et al., 2018) and with a recent large-scale survey that ranked the North African coast among the most metal-enriched areas for *P. lividus* in the Mediterranean (Yahiaoui Bouiba et al., 2024).

Table 9. Concentrations of cadmium, lead, copper, zinc and mercury (mg kg^{-1}) reported for *Mytilus galloprovincialis* and *Paracentrotus lividus* in recent surveys of the Mediterranean and adjacent seas, compared with the present study

Species	Location (period)	Tissue	Basis	Cd	Pb	Cu	Zn	Hg	Reference
<i>M. galloprovincialis</i>	Oran, Arzew and Mostaganem ports, Algeria (2025)	Whole soft tissue	dw	0.31–1.13	1.09–3.72	3.77–12.80	68.2–225.8	0.050–0.161	This study
<i>M. galloprovincialis</i>	Al Hoceima, Morocco (2018)	Soft tissue	dw	0.81 (mean)	n.d.	n.d.	159.9 (mean)	n.d.	Azizi et al., 2021a
<i>M. galloprovincialis</i>	Moroccan Mediterranean coast (2023)	Soft tissue	dw	0.001–0.77	0.05–0.60	1.27–6.70	24.4–190.2	n.d.	El Boudammoussi et al., 2024
<i>P. lividus</i>	Oran, Arzew and Mostaganem ports, Algeria (2025)	Whole soft tissue	dw	0.19–0.72	0.66–2.47	4.57–16.41	27.1–94.9	0.026–0.105	This study
<i>P. lividus</i>	Peniche, Portugal (2017)	Gonads ^a	dw	< LOD–1.20	0.43–2.77	4.42–11.95	93.1–402.6	n.d.	Sarly et al., 2023
<i>M. galloprovincialis</i>	Southern Marmara Sea, Türkiye	Soft tissue ^a	ww	0.21–0.33	0.28–0.45	1.79–3.11	55.7–94.3	0.027–0.053	Hacısalıhoğlu, 2025
<i>M. galloprovincialis</i>	Bulgarian Black Sea	Soft tissue ^a	ww	0.28–0.74	0.07–0.41	0.38–2.72	14.2–25.0	n.d.	Peycheva et al., 2023
<i>P. lividus</i>	Portuguese coast (2020–2021)	Gonads ^a	ww	n.d.	0–2.59	0.49–1.07	40–142	n.d.	Mamede et al., 2022

Note: dw – dry weight; ww – wet weight; n.d. – not determined in the cited study; LOD – limit of detection. Present-study values are the ranges of the composite samples over all sites and months. Published values are the ranges of the site or seasonal means given by the authors (Peniche: ranges of monthly means per shore, and per shore and sex for cadmium and zinc); single values are annual means. Dry- and wet-weight values are not directly comparable; ^a rows differing from the present study in tissue or weight basis, listed for reference only.

Condensing the five elements into a single index gave the same picture and made the magnitude of the contrast explicit: the overall burden at Oran was about 1.7 times that at Mostaganem in both species. The index is a relative summary score, not a measure of pollution in its own right: condensing five concentrations into one number necessarily discards the identity and relative weight of the individual elements, which is why the element-by-element results remain the primary evidence. The geometric mean was preferred because it limits the influence of zinc, the most abundant element, and is the form applied in recent Mediterranean surveys of these species (El Idrissi et al., 2023; El Mourabit et al., 2024). Index values are compared only within this study and are not transferable to surveys using a different suite of elements.

Complementary information from the two sentinel species

The two species agreed on the ranking of sites but differed markedly in what they accumulated, and this dissociation is analytically useful. Higher cadmium, lead, zinc and mercury in *M. galloprovincialis* is the expected consequence of suspension feeding, which passes large volumes of water and suspended particles across the gill and digestive gland and delivers both the dissolved and the particle-bound fraction of these elements (Kapranov et al., 2023; Peycheva et al., 2023). The very large zinc contrast is consistent with the high zinc content typical of bivalve soft tissue, where the element is abundant and under homeostatic control (Abbassi et al., 2021).

The reversal for copper, which was higher in the urchin, points to a different pathway. Grazing echinoids ingest benthic algae and associated surface sediment, and their tissues have been shown to respond to sediment-bound elements and to local substrate quality (El Idrissi et al., 2023; González-Delgado et al., 2024). At the urban station of Oran, copper, like the other elements, remained associated with turbidity and particulate load after adjustment for season, which is coherent with an exposure route mediated by resuspended particles and biofilm, although this association is correlative and station-specific. The species $\times$ station interaction adds that the excess of the mussel for cadmium, lead, zinc and mercury widened at the more contaminated stations, whereas the copper reversal was of constant magnitude, a pattern

compatible with a larger particle-bound supply of the four elements to the suspension feeder at Oran. This divergence supports the argument that a grazer and a suspension feeder should be used together rather than interchangeably, since each reports preferentially on a different compartment (Garcês and Pires, 2023; Klaoudatos et al., 2022).

Seasonal dynamics

Season accounted for a significant but secondary share of the variance, with a summer maximum roughly a third above the winter minimum for all elements. Several non-exclusive mechanisms are compatible with this pattern. Higher summer temperature raises filtration and metabolic rates and therefore the rate at which metals are taken up, and the negative association between metal burdens and dissolved oxygen observed at Mostaganem is consistent with warmer, more stratified and less renewed water in summer; these within-station associations share a seasonal component, however, and do not by themselves identify a mechanism of metal input. Reduced freshwater input and weaker flushing in the dry season also concentrate contaminants in and around harbour basins. Seasonal variation of comparable amplitude has been documented in Mediterranean mussels (Peycheva et al., 2023) and in *P. lividus* (El Idrissi et al., 2020; Ouchene et al., 2021), although the timing of maxima differs among studies, in part because reproductive cycles and hydrological regimes differ between regions.

Because the seasonal amplitude measured here was small relative to the among-site range, seasonality does not compromise the detection of the spatial gradient. For the three metals with the steepest gradient, the summer maximum at the least contaminated port remained below the winter minimum at the most contaminated one.

Biometric patterns and the size confounder

Both species displayed tight length–weight relationships, close to isometric in the mussel and moderately negatively allometric in the urchin, values that fall within the range reported for Mediterranean populations of these taxa (Grković et al., 2020; Klaoudatos et al., 2022). This internal coherence supports the use of the derived condition indices for comparison among sites.

A notable pattern is that individuals from the least contaminated station were consistently the

largest, and urchins there also showed the highest gonadosomatic index, whereas the condition indices differed little or not at all among sites. Two interpretations must be kept apart. Reduced growth and gonadal investment under chronic metal exposure is a plausible and documented response in both taxa (Shen et al., 2022; Ouchene et al., 2021), but the present design cannot establish it, because the stations also differ in trophic conditions, hydrodynamics, substrate and harvesting pressure, any of which can drive size structure independently of contamination. The association reported here is therefore descriptive and no causal link between metal burden and condition is claimed.

The size difference also has a methodological consequence that deserves emphasis. Because animals from the most contaminated ports were the smallest, and because metal concentrations expressed per unit dry weight often decrease with increasing body size through growth dilution, part of the among-site contrast in concentration could be amplified by the size difference (Kapranov et al., 2023). The analysis of covariance addressed this point directly: adjusting for mean body size roughly halved the F statistic of the site effect but left it highly significant for every element in both species, and the size covariate was significant only for cadmium, zinc and mercury in the mussel, with small negative slopes, and the homogeneity of slopes was supported except for lead in the mussel. The gradient is therefore not an artefact of size structure, and the direction of the ranking is further corroborated by the independent physico-chemical measurements; the adjustment does not, however, compensate for the absence of spatial replication within ports. The residual size effect in the mussel nevertheless argues for size-matched sampling in future monitoring at these sites.

Scope of inference and limitations of the design

Each port was represented by one fixed station, and the three composite samples collected there each month are subsamples of that station rather than independent spatial replicates; consecutive months are repeated observations of the same station. The effective spatial replication for a comparison of ports is therefore one station per port, and the differences reported here are differences among three harbour stations, not estimates of the mean contamination of each port. The

block and mixed-model analyses account for the temporal dependence and remove within-station subsampling from the error term, but cannot create spatial replication. Because the ranking was reproduced by two species, five elements and twelve monthly campaigns, was corroborated by independent water measurements, it is unlikely to be an artefact of the particular station chosen in each port; this cannot be tested with the present design, and generalisation from station to port requires several independent stations per port. Because the stations are on the seaward face of the breakwaters, they record the contamination of the immediate surroundings of each harbour rather than of its enclosed basin, where burdens are likely to be higher.

Other limitations follow from the scope of the measurements: metal sources were not characterised directly, tissue moisture was not determined, the reference materials are of bivalve origin so that a matrix bias for the urchin cannot be excluded, quality control is documented as a batch acceptance range rather than element-by-element means and no formal measurement uncertainty was estimated, biometric data exist only as composite means, and the within-station correlations describe covariation, not causation.

Implications for coastal biomonitoring

The results identify the Oran and Arzew harbour stations as the priority sites for sustained surveillance on this stretch of coast, and the Mostaganem station as a usable local reference against which change can be measured, within the scope of inference set out above. Three operational conclusions follow. First, because the spatial ranking was stable across seasons, an annual campaign at a fixed season is sufficient to track relative change among sites, provided the season is held constant. Second, because the two species diverge for copper, monitoring both is preferable to monitoring either alone; the mussel is the more sensitive reporter for cadmium, lead, zinc and mercury, and the urchin adds information on the sediment-associated copper pool. Third, the coupling of tissue burdens with turbidity and particulate load at the urban station of Oran suggests that routine nephelometric monitoring may serve as an inexpensive proxy for periods of elevated exposure risk at urban harbours, a hypothesis to be tested with spatially replicated sampling.

These findings also connect to concerns about seafood safety in the region, since *P. lividus* gonads and mussels are both consumed locally, and recent Algerian work has documented trace metal burdens of regulatory relevance in fishery products (Kaddour et al., 2022; Haddidi et al., 2026). Mitigation of the industrial and urban inputs that sustain these gradients, whether through effluent treatment or through harbour management, remains the condition for any durable improvement (Fadli et al., 2024; Yehdih et al., 2022). The present dataset provides the quantitative baseline against which such measures can be evaluated. It complements comparable regional surveys of mussels and sediments (El Boudammoussi et al., 2024; Azizi et al., 2023). Caged-organism and multi-element approaches developed elsewhere in the Mediterranean offer a further point of comparison (Barre et al., 2024; Azizi et al., 2021b). Metallothionein induction and shell matrix analysis offer further avenues for refining exposure assessment in mussels (Abbassi et al., 2022; Martinović et al., 2023). Controlled exposure to metal mixtures under laboratory conditions provides a third, mechanistic line of evidence (Boudjema et al., 2022; Azizi et al., 2021a).

A regulatory reading of the present values is not attempted. The European Union sets maximum levels in bivalve molluscs of 1.0, 1.5 and 0.5 mg kg⁻¹ wet weight for cadmium, lead and mercury respectively, and no specific level exists for echinoderms (European Commission, 2023). Compliance with such limits can only be assessed on a wet-weight basis, using the measured moisture content of each sample to convert the dry-weight concentrations; moisture was not determined in this campaign, so no statement on compliance is made for any element. Determination of tissue moisture and of wet-weight lead in mussels from the Oran and Arzew stations is the most pressing complement to the present dataset, lead being the element found above the regulatory level in urchin gonads at two Algerian sites in a recent survey (Yahiaoui Bouiba et al., 2024).

CONCLUSIONS

A twelve-month survey of two sentinel invertebrates at one station in each of three ports of the Algerian west coast established a consistent spatial gradient of tissue contamination by

cadmium, lead, copper, zinc and mercury among the three stations, ordered Oran > Arzew > Mostaganem. Every pairwise difference between stations was statistically significant for every element, in both species, and the ordering was reproduced in all four seasons. Because each port was represented by a single station, these conclusions apply to the three stations sampled and not to the ports as a whole.

Station was the dominant source of variation in metal burdens in every model fitted, and its effect persisted on station–month means with month as a blocking factor, in mixed models, under heteroscedasticity-robust and rank-based tests and after adjustment for body size. Season contributed a significant but smaller share, with concentrations about one third higher in summer than in winter, and no interaction between site and season was detected, so the spatial pattern was stable throughout the annual cycle.

The two species provided complementary rather than redundant information. The mussel accumulated more cadmium, lead, zinc and mercury, whereas the urchin accumulated more copper, indicating that suspension feeding and grazing sample different fractions of the available metal pool.

Length–weight relationships were tight in both species, close to isometric in the mussel and negatively allometric in the urchin. Individuals from the least contaminated port were the largest and, in the urchin, showed the highest gonadal investment, although the design does not allow this association to be attributed to metal exposure rather than to concurrent differences in trophic and hydrodynamic conditions.

After adjustment for species and season and correction for multiple testing, tissue metal concentrations remained associated with turbidity and suspended particulate matter at the Oran station and with temperature and dissolved oxygen at the Mostaganem station, whereas no association persisted at Arzew; salinity and pH were unrelated to metal burdens everywhere. These associations describe covariation and do not identify a mechanism.

An integrated index combining the five elements into a single value per sample reproduced the same ordering of the three stations, the same summer maximum and the same absence of interaction between site and season, and it confirmed the higher overall burden of the mussel. As a relative summary score it reproduces the

spatial and seasonal ranking of the individual elements, while the element-by-element results remain the primary evidence.

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REFERENCES

- Abbassi, M., Aboudlou, L., Charioui, I., Nadir, M., Agnaou, M., Achahour, O., Bari, S., Jebali, J., Hamoudi, B., Bani, M., Kaaya, A. (2021). Assessment of Cd, Cu, Zn and Ni bioaccumulation and metallothionein concentrations in *Mytilus galloprovincialis* in Sidi Ifni coast (South Atlantic of Morocco). *International Journal of Agriculture, Environment and Bioresearch*, 6(3), 103–115. <https://doi.org/10.35410/IJAEB.2021.5632>
- Abbassi, M., Aboudlou, L., Charioui, I., Achahour, O., Ouaisa, K., Zine, H., Kaaya, A. (2022). Study of seasonal variations of the condition indices in the mussel *Mytilus galloprovincialis*: Relationship with physico-chemical parameters, biomarkers and metals in the region of Sidi Ifni (South of Morocco). *International Journal of Agriculture, Environment and Bioresearch*, 7(4), 181–200. <https://doi.org/10.35410/ijaeb.2022.5755>
- Azizi, G., Layachi, M., Akodad, M., Martín-García, A.I., Yáñez-Ruiz, D.R., Baghour, M., Ait Hmeid, H., Gueddari, H., Moumen, A. (2021a). Bioaccumulation and health risk assessment of trace elements in *Mytilus galloprovincialis* as sea food in the Al Hoceima coasts (Morocco). *E3S Web of Conferences*, 240, 01002. <https://doi.org/10.1051/E3SCONF/202124001002>
- Azizi, G., Akodad, M., Layachi, M., Ait Hmeid, H., Baghour, M., Gueddari, H., Riouchi, O., Moumen, A. (2021b). Biomonitoring study of trace metals (Al, As, Li) in mussels from Al Hoceima coastline of Moroccan Mediterranean Sea. *E3S Web of Conferences*, 314, 07001. <https://doi.org/10.1051/E3SCONF/202131407001>
- Azizi, G., Akodad, M., Layachi, M., Baghour, M., Said, B., Ait Hmeid, H., Ngadi, H., Gueddari, H., Esseffar, S., Riouchi, O., Moumen, A. (2023). Bio-monitoring heavy metals (Cu, Li and Mn) in the Marchica lagoon of Morocco using *Mytilus galloprovincialis*. *E3S Web of Conferences*, 364, 01007. <https://doi.org/10.1051/e3sconf/202336401007>
- Barre, A., Briand, J.F., Vaccher, V., Briant, N., Briand, J.M., Dormoy, B., Boissery, P., Bouchoucha, M. (2024). A comparative biomonitoring study of trace metals and organic compounds bioaccumulation in marine biofilms and caged mussels along the French Mediterranean coast. *Environmental Pollution*, 363, 125239. <https://doi.org/10.1016/j.envpol.2024.125239>
- Belabed, B.E., Meddour, A., Samraoui, B., Chenchouni, H. (2017). Modeling seasonal and spatial contamination of surface waters and upper sediments with trace metal elements across industrialized urban areas of the Seybouse watershed in North Africa. *Environmental Monitoring and Assessment*, 189(6), 265. <https://doi.org/10.1007/s10661-017-5968-5>
- Boudjema, K., Badis, A., Moulai-Mostefa, N. (2022). Study of heavy metal bioaccumulation in *Mytilus galloprovincialis* (Lamarck 1819) from heavy metal mixture using the CCF design. *Environmental Technology & Innovation*, 25, 102202. <https://doi.org/10.1016/j.eti.2021.102202>
- Castrec, J., Pillet, M., Receveur, J., Fontaine, Q., Le Floch, S., Churlaud, C., Lejeune, P., Gobert, S., Thomas, H., Marengo, M. (2023). Active and passive biomonitoring of trace elements, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls in small Mediterranean harbours. *Marine Pollution Bulletin*, 187, 114578. <https://doi.org/10.1016/j.marpolbul.2023.114578>
- El Boudammoussi, M., El Hammoudani, Y., Reklou, K., Dimane, F., Haboubi, K. (2024). Trace element correlations in mussels and sediments on the Moroccan Mediterranean coast. *Ecological Engineering & Environmental Technology*, 25(7), 106–117. <https://doi.org/10.12912/27197050/188025>
- El Idrissi, O., Marengo, M., Aiello, A., Gobert, S., Pasqualini, V., Ternengo, S. (2020). Seasonal change in trace element concentrations of *Paracentrotus lividus*: Its use as a bioindicator. *Ecological Indicators*, 112, 106063. <https://doi.org/10.1016/j.ecolind.2019.106063>
- El Idrissi, O., Ternengo, S., Monnier, B., Lepoint, G., Aiello, A., Bastien, R., Lourkisti, R., Bonnin, M., Santini, J., Pasqualini, V., Gobert, S. (2023). Assessment of trace element contamination and effects on *Paracentrotus lividus* using several approaches: Pollution indices, accumulation factors and biochemical tools. *Science of The Total Environment*, 869, 161686. <https://doi.org/10.1016/j.scitotenv.2023.161686>

13. El Mourabit, Y., Hasni, M., Agnaou, M., Nadir, M., Abou Oualid, J., Moukrim, A., Ait Alla, A. (2024). Assessment of trace metal bioaccumulation in *Mytilus galloprovincialis* of the Central Atlantic Ocean after installation of treatment sewage facilities. *Chemosphere*, 348, 140730. <https://doi.org/10.1016/j.chemosphere.2023.140730>
14. European Commission. (2023). Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. *Official Journal of the European Union*, L 119, 103–157. <http://data.europa.eu/eli/reg/2023/915/oj>
15. Fadli, M.I., Bustan, M.D., Haryati, S. (2024). Reducing pollutants in coal wastewater with *Moringa oleifera* and natural biocoagulants combination. *Ecological Engineering & Environmental Technology*, 25(8), 232–244. <https://doi.org/10.12912/27197050/189806>
16. Garcês, A., Pires, I. (2023). The guardians of the sea: echinoderms as sentinels of marine pollution. *Toxicology International*, 29(4), 541–552. <https://doi.org/10.18311/ti/2022/v29i4/30487>
17. González-Delgado, S., Lozano-Bilbao, E., Hardisson, A., Paz, S., González-Weller, D., Rubio, C., Gutiérrez, Á.J. (2024). Metal concentrations in echinoderms: Assessing bioindicator potential and ecological implications. *Marine Pollution Bulletin*, 205, 116619. <https://doi.org/10.1016/j.marpolbul.2024.116619>
18. Grković, N., Teodorović, V., Djordjević, V., Karabasil, N., Stajković, S., Vasilev, D., Zuber Bogdanović, I., Janković, S., Velebit, B., Dimitrijević, M. (2020). Biochemical composition and biometric parameters of *Mytilus galloprovincialis* from Boka Kotorska Bay in Southern Adriatic Sea. *Journal of the Hellenic Veterinary Medical Society*, 71(3), 2338. <https://doi.org/10.12681/JHVMS.25095>
19. Guendouzi, Y., Soualili, D.L., Boulahdid, M., Boudjellal, B. (2018). Biological indices and monitoring of trace metals in the mussel from the southwestern Mediterranean (Algeria): Seasonal and geographical variations. *Thalassas: An International Journal of Marine Sciences*, 34(1), 103–112. <https://doi.org/10.1007/s41208-017-0043-0>
20. Hacısalihoğlu, S. (2025). Heavy metal accumulation and human health risk in Mediterranean mussels from the Southern Marmara Sea. *Toxics*, 13(12), 1084. <https://doi.org/10.3390/toxics13121084>
21. Haddidi, I., Bouteldja, M., Malek, I., Zeghdoudi, F., Touati, H., Chelaghmia, M.L., Affoune, A.M., Boudalia, S. (2026). Trace metal concentrations in the edible tissues of fishery products: Human health risk assessment in the Guelma region (Algeria). *Ecological Engineering & Environmental Technology*, 27(7), 324–342. <https://doi.org/10.12912/27197050/224341>
22. Kaddour, A., Djellouli, F., Belhoucine, F., Alioua, A. (2022). Heavy metal bioaccumulation and genotoxicity in fish (*Merluccius merluccius*, Linnaeus, 1758) from the Western Algerian Mediterranean coast. *Applied Ecology and Environmental Research*, 20(6), 5361–5379. https://doi.org/10.15666/aeer/2006_53615379
23. Kapranov, S.V., Kozintsev, A.F., Bobko, N.I., Ryabushko, V.I. (2023). Elements in soft tissues of the young Mediterranean mussel *Mytilus galloprovincialis* Lam. 1819 collected in Sevastopol Bay (Crimea, Black Sea): Effects of age, sex, location, and principal morphometric parameters. *Animals*, 13(12), 1950. <https://doi.org/10.3390/ani13121950>
24. Klaoudatos, D., Tziantziou, L., Lolas, A., Neofitou, N., Vafidis, D. (2022). Population characteristics of the upper infralittoral sea urchin *Arbacia lixula* (Linnaeus, 1758) in Eastern Mediterranean (Central Greece): An indicator species for coastal water quality. *Journal of Marine Science and Engineering*, 10(3), 395. <https://doi.org/10.3390/jmse10030395>
25. Mamede, R., Duarte, I.A., Caçador, I., Tanner, S.E., Silva, M., Jacinto, D., Fonseca, V.F., Duarte, B. (2022). Elemental fingerprinting of sea urchin (*Paracentrotus lividus*) gonads to assess food safety and trace its geographic origin. *Journal of Food Composition and Analysis*, 114, 104764. <https://doi.org/10.1016/j.jfca.2022.104764>
26. Martinović, R., Joksimović, D., Perošević-Bajčeta, A., Čabarkapa, I., Ehrlich, H. (2023). Shell organic matrix (Conchix) of the Mediterranean mussel *Mytilus galloprovincialis* L. as the medium for assessment of trace metals in the Boka Kotorska Bay. *Applied Sciences*, 13(13), 7582. <https://doi.org/10.3390/app13137582>
27. Ouchene, H., Chahouri, A., Hafidi, N., Elouizgani, H., Hermas, J. (2021). Seasonal changes in gonad index, biochemical composition and heavy metal determination of sea urchin *Paracentrotus lividus* gonads from the South coast of Morocco. *Ocean Science Journal*, 56(4), 344–354. <https://doi.org/10.1007/S12601-021-00038-8>
28. Peycheva, K., Panayotova, V., Stancheva, R., Merdzhanova, A., Dobrev, D., Parrino, V., Cicero, N., Fazio, F., Licata, P. (2023). Seasonal variations in the trace elements and mineral profiles of the bivalve species, *Mytilus galloprovincialis*, *Chamelea gallina* and *Donax trunculus*, and human health risk assessment. *Toxics*, 11(4), 319. <https://doi.org/10.3390/toxics11040319>
29. Rouane-Hacene, O., Boutiba, Z., Benaissa, M., Belhaouari, B., Francour, P., Guibbolini-Sabatier, M.E., Faverney, C.R.D. (2018). Seasonal assessment of biological indices, bioaccumulation, and bioavailability of heavy metals in sea urchins *Paracentrotus*

- lividus* from Algerian west coast, applied to environmental monitoring. *Environmental Science and Pollution Research*, 25(12), 11238–11251. <https://doi.org/10.1007/s11356-017-8946-0>
30. Sarly, M.S., Pedro, C.A., Bruno, C.S., Raposo, A., Quadros, H.C., Pombo, A., Gonçalves, S.C. (2023). Use of the gonadal tissue of the sea urchin *Paracentrotus lividus* as a target for environmental contamination assessment. *Environmental Science and Pollution Research*, 30(38), 89559–89580. <https://doi.org/10.1007/s11356-023-28472-2>
31. Shen, H., Nzabanita, D., Grist, S., Miranda, A., Long, S.M., Nugegoda, D. (2022). Trends in bioaccumulation and metabolite profiles in Mediterranean mussels with sub lethal exposure to mixtures of trace metals. *Journal of Environmental Chemical Engineering*, 10(1), 106825. <https://doi.org/10.1016/j.jece.2021.106825>
32. Yahiaoui Bouiba, S., Bendimerad, M.E.A., Rouane-Hacene, O., Boualga, A., Richir, J. (2024). Metallic trace element dynamics in *Paracentrotus lividus* from Algeria: Environmental large-scale survey and human health risk assessment. *Science of The Total Environment*, 915, 169492. <https://doi.org/10.1016/j.scitotenv.2023.169492>
33. Yehdhih, M., El Hadi, H., Baghdad, B., Chakiri, S., Hamoud, A., Zerdeb, A., Moussa, K. (2022). Assessment of heavy metals pollution in the marine sediments of the Lévrier Bay (Nouadhibou, Mauritania). *Ecological Engineering & Environmental Technology*, 23(5), 84–90. <https://doi.org/10.12912/27197050/151641>