LOOK ACADEMIC PUBLISHERS
OPEN ACCESS

INTERNATIONAL JOURNAL OF ENVIRONMENTAL & SCIENCE EDUCATION
2017, VOL. 12, NO. 10, 2347-2361

Contamination levels of metals (Cu, Cr, Cd and Pb) in *Patella rustica* from the Moroccan Atlantic coast

Lalla Meryem IDRISSI AZZOUZI[a*], Rachid Ben Aakame[b], Abdellah El Abidi[b], Mariam Naciri[c]

[a]Laboratory of Virology, National Institute of Hygiene and Laboratory of Biodiversity, Ecology and Genome, University Mohamed V of Rabat, School of Sciences, MOROCCO;

[b]Laboratory of Hydrobiology, Toxicology, Industrial and Environmental Hygiene, National Institute of Hygiene, Rabat, MOROCCO;

[c]Laboratory of Biodiversity, Ecology and Genome, University Mohamed V of Rabat, School of Sciences, MOROCCO

ABSTRACT

This study was carried out to determine the levels of some metal including copper (Cu), chromium (Cr), cadmium (Cd) and lead (Pb) in tissues of the mollusca gastropoda limpet (*Patella rustica*), using the standard atomic absorption spectrometry (AAS) technique after mineralization method. These samples were collected from three different locations (Yacoub Al Mansour, Harhoura and Guy ville) of Moroccan Atlantic coast during four seasons (winter, spring, summer and autumn). The average ranges of elements concentrations measured in samples were: Yacoub Al Mansour coast (Cu: 0.46-2.19 µg/g; Cr: 0,60-2,21 µg/g; Cd: 0,32-1,06 µg/g and Pb: 0,47-1,30 µg/g), Harhoura coast: (Cu: 0.81-3.17 µg/g; Cr: 0,94-2,5 µg/g; Cd: 0,47-0,95 µg/g and Pb: 0,76-1,42 µg/g) and Guy ville coast (Cu: 1.24-4.14 µg/g; Cr: 0,87-3,98 µg/g; Cd: 0,56-1,18 µg/g and Pb: 1,08-2,13 µg/g). During all seasons and from different sampling sites, the results obtained indicate that metals concentration in *Patella rustica* tissues were distributed differently .

KEYWORDS
Contamination assessment, *Patella rustica*, Gastropod molluscs, Metals, Moroccan Atlantic coast.

ARTICLE HISTORY
Received 11 September 2017
Revised 30 October 2017
Accepted 19 December 2017

Introduction

Environmental pollution by metals is constantly increasing. Therefore, the environmental protection has become a currently major concern for every country in the world. The metal elements are considered as critical contaminants in the

CORRESPONDENCE Lalla Meryem IDRISSI AZZOUZI ✉ midrissi28@yahoo.fr

environment, due to their high potential to enter and transfer in food chains and can be accumulated in the soft tissues of different marine organisms like shellfish. Subsequently, this accumulation establishes a great danger for the human consumption when the concentration exceeds certain threshold of acceptability (Bradl, 2005; He et al., 2005; Tchounwou et al., 2012).

The Moroccan Atlantic coast extends for about 3500 km. It has considerable biological diversity and fish production (Snoussi et al., 2008; Chaibi & Sedrati, 2009). This coastline is threatened with several types of pollution. Indeed, many chemical pollutants including metal elements and organic contaminants are rejected in this coastal system by industrial, agricultural and anthropogenic activities (Maanan, 2008; Maanan et al., 2015). Metals such as cadmium (Cd), lead (Pb), chromium (Cr), mercury (Hg), copper (Cu), zinc (Zn), manganese (Mn) and nickel (Ni)… were among these rejections and constitute a group of the most important chemical pollutants. These elements cause a considerable harm to the environment (Bradl, 2005; Tchounwou et al., 2012; Ben Aakame et al., 2014).

Heavy metals are naturally occurring elements in the environment that have a relatively high atomic weight and density compared to water. Their concentrations in the marine environment are the results of both natural sources and anthropogenic activities (Glasby et al., 2004; Maanan et al., 2004; Singh et al., 2011; Tchounwou et al., 2012; Wani et al., 2017). The accumulation of metal elements in the aquatic environment affects various organisms (Baby et al., 2010). These chemical agents are divided in essential elements that are required to support biological activities and non-essential metals with an unknown biological function. The latter being toxic for organisms when subject to high concentrations, it induces a multiple organ damage even at lower levels of exposure (Tchounwou et al., 2012; Jaishankar et al., 2014).

Gastropods molluscs are filter feeders and thus obtain heavy metals from water, food and ingestion of inorganic materials (Singh et al., 2014). It is well known that these organisms accumulate metallic and organic pollutants in their tissues responding essentially to the fraction existing in the marine environment (Bergasa et al., 2007). In this study, gastropods were employed as bio-indicator to determine the effect of marine pollution. These organisms were considered as appropriate indicators since they are available all the year, present in almost all coastal areas and easily collected (Yuzereroglu et al., 2010; Richir and Gobert 2016). Linnaeus in 1758 described the taxon *Patella rustica* from the morphological characters in order to identify this species collected from an unknown locality (Sa-Pinto et al., 2010).

Patella rustica and other gastropods are frequently used as sentinel organisms in monitoring programs in coastal environments due to their ability to accumulate metal elements in their tissues (Bergasa et al., 2007). In this research, we examined the concentrations level of Cu, Cr, Cd and Pb in the tissues of *P. rustica* during four seasons from three different sites of Moroccan Atlantic coast in Rabat-Sale region.

The aim of this study was to assess the contamination level of metals in the aquatic environment and evaluate the public health risk associated with consumption of contaminated gastropod molluscs.

Data obtained were used in the future to assess the toxicological risk due to the consumption of *P. rustica.* Thus, four metals (copper, chromium, cadmium and lead) were selected for contamination assessment of *P. rustica*, as well as a comparison between the metals concentration detected in their tissues during four seasons.

Material and methods

Study Area

The sampling sites were situated in Rabat-Sale region in Morocco (Fig. 1). This region covers an area of 18.194 km², with a population around 4.581.000. This area belongs to the Mediterranean climate marked by two main seasons softened by oceanic influences. The average temperatures are around 22°C for the warmer months (July to September) and 12°C for the colder months (December and January). Concerning the annual rainfall is in average more than 550 mm/year (Idrissi Azzouzi et al., 2017).

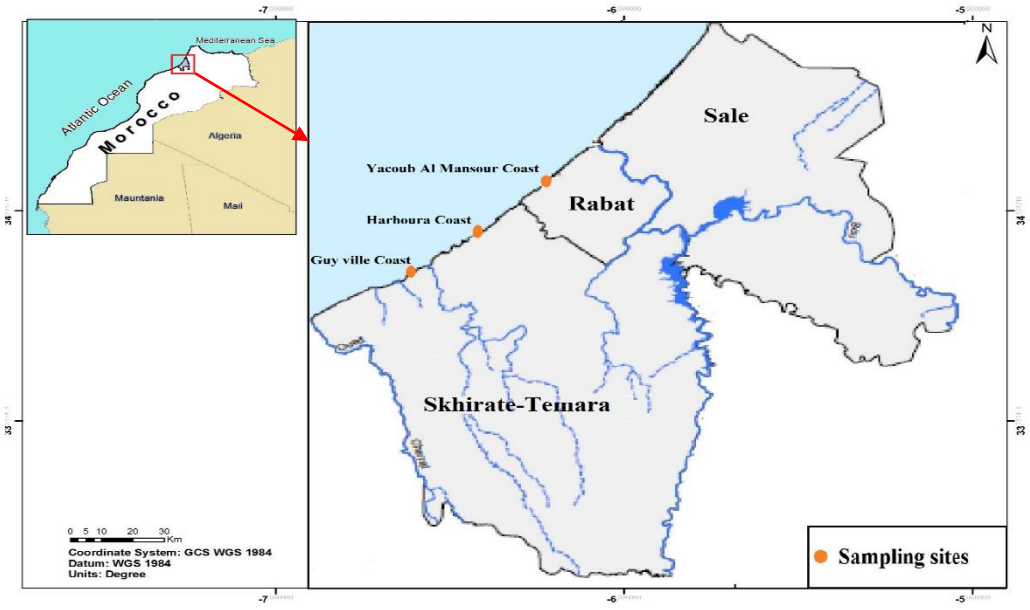


Figure 1. Map of Morocco coast showing the sampling sites.

Sample collection

Between February 2015 and February 2016 a total of 120 shellfish of *Patella rustica* common name rustic limpet were collected monthly and seasonally, from three wild populations (Yacoub Al Mansour, Harhoura and Guy ville) of Moroccan Atlantic coast in Rabat-Sale region. These locations receive large quantities of untreated or partially treated domestic wastewater.

The samples were collected by scalpel at rocky shores of three different intertidal locations from the Rabat-Sale region. The specimens collected were onsite waste, stored in polyethylene bags and frozen at -20°C until analysis.

Sample preparation

The sample was partially thawed at room temperature; the soft (edible) parts were dissected separately from the shells using a clean stainless steel scalpel and then washed with deionized water. The samples washed were drained in an incubator at 65°C for 48 h until complete drying and then ground using porcelain mortar. For this purpose, the dried samples were prepared to measure their concentrations.

Several studies have been based on the analysis of heavy metals in the storage organs (liver, kidney, spleen). However, these organs can accumulate very strongly a metal, without being significant in the surrounding environment; therefore, in order to assess the risks incurred by the consumers of gastropod molluscs such as *Patella rustica*, we analyzed primarily the edible parts that mean all the soft tissues (Nakhle, 2003).

Mineralization

In total, ten samples from each location in each season means forty samples in every station. The samples were analyzed in order to determine copper (Cu), chromium (Cr), cadmium, (Cd) and lead (Pb) in the tissues of *Patella rustica*. This determination was carried out after mineralization of the samples.

In the clean digestion bombs containing of *P. rustica* tissues, 4 mL of nitric acid (HNO_3) 65% (Merck) and 1 mL of oxygenated water (H_2O_2) 30% (Merck) were added. The samples were kept overnight for predigesting, the next day they were placed in sand bath at 120°C for 4 h until the solution became clear. After cooling, we diluted our samples to reach a final volume of 30 ml with ultra-pure water appropriately in the range of standards that were prepared from standard solution of the metals (Conti et al., 2003; Kelepertzis et al., 2013).

Analysis of heavy metals

Determination

After dilution, the metal concentrations in the samples tissues were measured by Atomic Absorption Spectrometry with Graphite Furnace (AAS-GF) type (Varian 240 Zeeman) and presented as microgram metal/gram dry weight. The instrument was calibrated with metal standard solutions (1g/L) prepared by dilution (Cravo et al., 2005; Collado et al., 2006; Nakhle et al., 2006; Bergasa et al., 2007).

The accuracy and precision of this methodology were tested by using a separate comparative study of a standard reference material (IAEA-MEL, 2016-01-TE). The agreement between the results for the reference biological material certified values was satisfactory and proving a good repeatability of the method (Table 1). The recovery values of metals analysis were between 85% and 96% and the analytical methods were carried out using triplicate samples.

Table 1. Analysis of certified reference material (IAEA-MEL, 2016-01-TE): certified values and found values (means± relative standard deviation) (in µg/g of dry weight).

Metals	Certified	Found
Cu	2,860±0,300	2,490±0,270
Cr	7,830±1,100	7,750±1,090
Cd	0,033±0,004	0,030±0,001
Pb	0,648±0,074	0,587±0,037

Statistical methods

The concentrations (mean and standard deviation) of heavy metals (Cu, Cr, Cd and Pb) were calculated for overall registered data. The standard deviations refer to the variability within different replicates. In order to verify the differences in the heavy metal levels in *Patella rustica* during the four seasons a non-parametric statistical method (the Student t test) was used for determination of significant differences ($p < 0.05$).

The Mann-Whitney U test was conducted to test the significance of the differences in the metal content between the three sampling sites. The level of significance was set at $p < 0.01$.

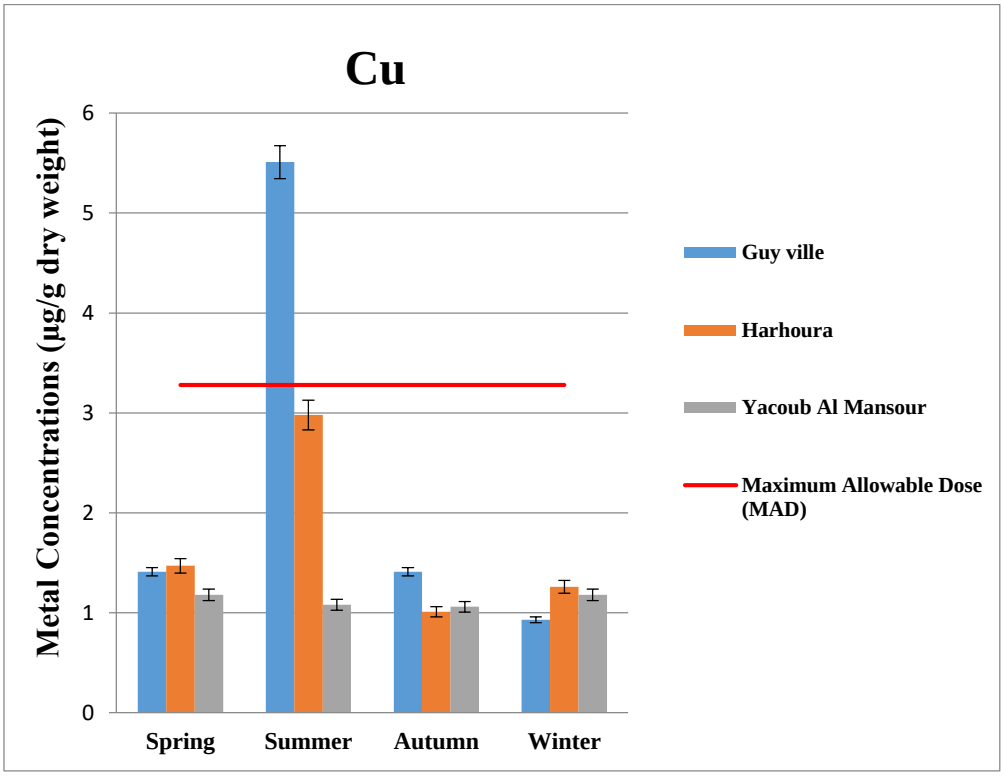
All analysis was performed using the package R version 3.4.1 software.

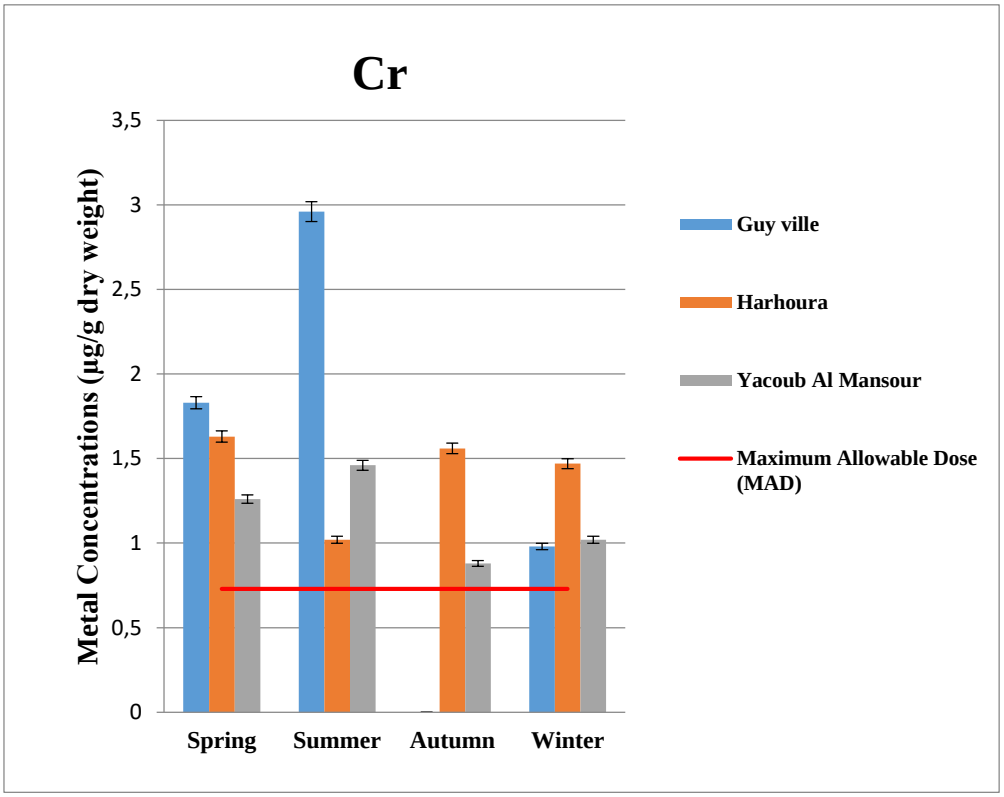
Results and discussion

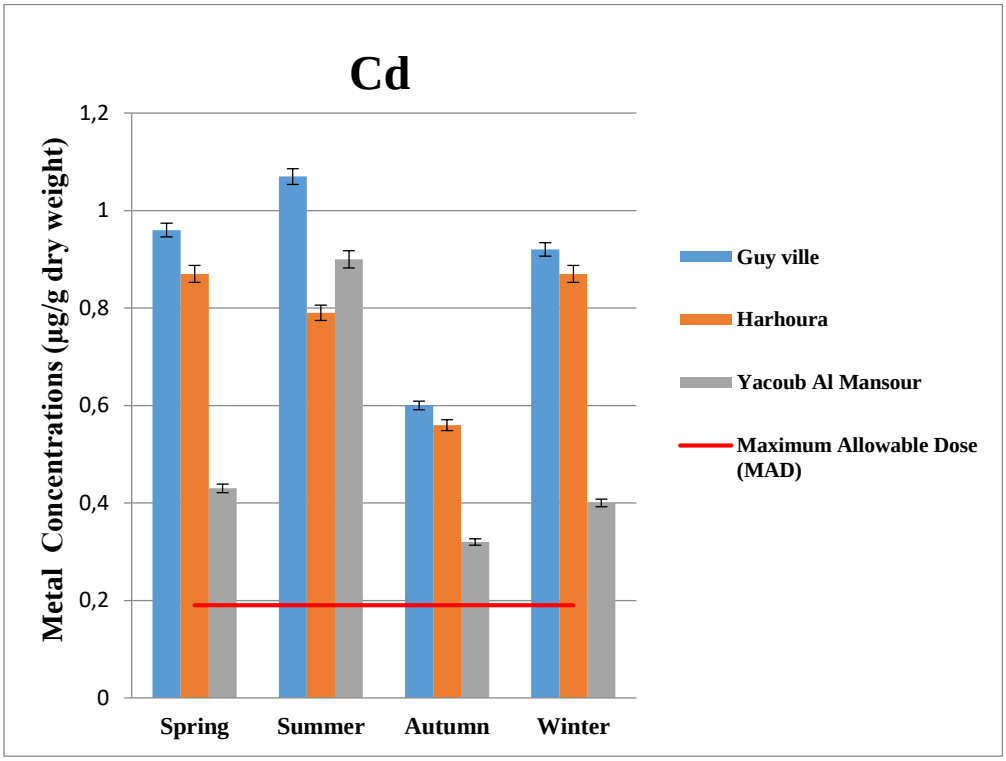
In Morocco, the bivalve molluscs such as *Mytilus galloprovincialis* were already used to study the chemical contamination. However, up until now, no study has been undertaken to assess the metal contaminations in the gastropod molluscs (*Patella rustica*). The results obtained showed that the average concentrations of the metals in *Patella rustica* tissues from all stations varied significantly and decreased in the order of Cu>Cr>Pb>Cd. The average ranges were as follows: Yacoub Al Mansour coast (Cu: 0.46-2.19 µg/g; Cr: 0,60-2,21 µg/g; Cd: 0,32-1,06 µg/g and Pb: 0,47-1,30 µg/g), Harhoura coast: (Cu: 0.81-3.17 µg/g; Cr: 0,94-2,5 µg/g; Cd: 0,47-0,95 µg/g and Pb: 0,76-1,42 µg/g) and Guy ville coast (Cu: 1.24-4.14 µg/g; Cr: 0,87-3,98 µg/g; Cd: 0,56-1,18 µg/g and Pb: 1,08-2,13 µg/g). From different sampling sites, the results of the present study revealed that metal concentrations in *Patella rustica* tissues were distributed differently (Table 2 and Fig. 2).

Table 2. Mean concentrations ± relative standard deviation (RSD) values (µg/g of dry weight) of metals in *Patella rustica* from different stations during four seasons.

Elements	Stations	Seasons			
		Spring	Summer	Autumn	Winter
Cu	Yacoub Al Mansour coast	1,18±0,05	1,08± 0,03	1,06± 0,05	1,18± 0,06
	Harhoura coast	1,47±0,02	2,92± 0,14	1,01± 0,03	1,26± 0,02
	Guy ville coast	1,41±0,01	5,51± 0,45	1,41± 0,04	0,93± 0,06
Cr	Yacoub Al Mansour coast	1,26±0,06	1,46±0,04	0,88±0,02	1,02±0,03
	Harhoura coast	1,63±0,07	1,02±0,02	1,56±0,04	1,47±0,02
	Guy ville coast	1,83±0,09	2,96±0,16	1.68±0,06	0,98±0,02
Cd	Yacoub Al Mansour coast	0,43±0,01	0,90±0,04	0,32±0,01	0,40±0,01
	Harhoura coast	0,87±0,03	0,79±0,02	0,56±0,03	0,87±0,03
	Guy ville coast	0,96±0,07	1,07±0,02	0,60±0,01	0,92±0,04
Pb	Yacoub Al Mansour coast	0,50±0,01	1,27±0,01	0,73± 0,03	0,86±0,02
	Harhoura coast	0,97±0,03	1,01±0,02	0,93±0,04	0,97±0,05
	Guy ville coast	1,16±0,06	1,97±0,08	1,19±0,07	1,01±0,02


Cu
Metal Concentrations (µg/g dry weight)
0
1
2
3
4
5
6
Spring
Summer
Autumn
Winter
Guy ville
Harhoura
Yacoub Al Mansour
Maximum Allowable Dose (MAD)


Cr
Metal Concentrations (µg/g dry weight)
0
0,5
1
1,5
2
2,5
3
3,5
Spring
Summer
Autumn
Winter
Guy ville
Harhoura
Yacoub Al Mansour
Maximum Allowable Dose (MAD)



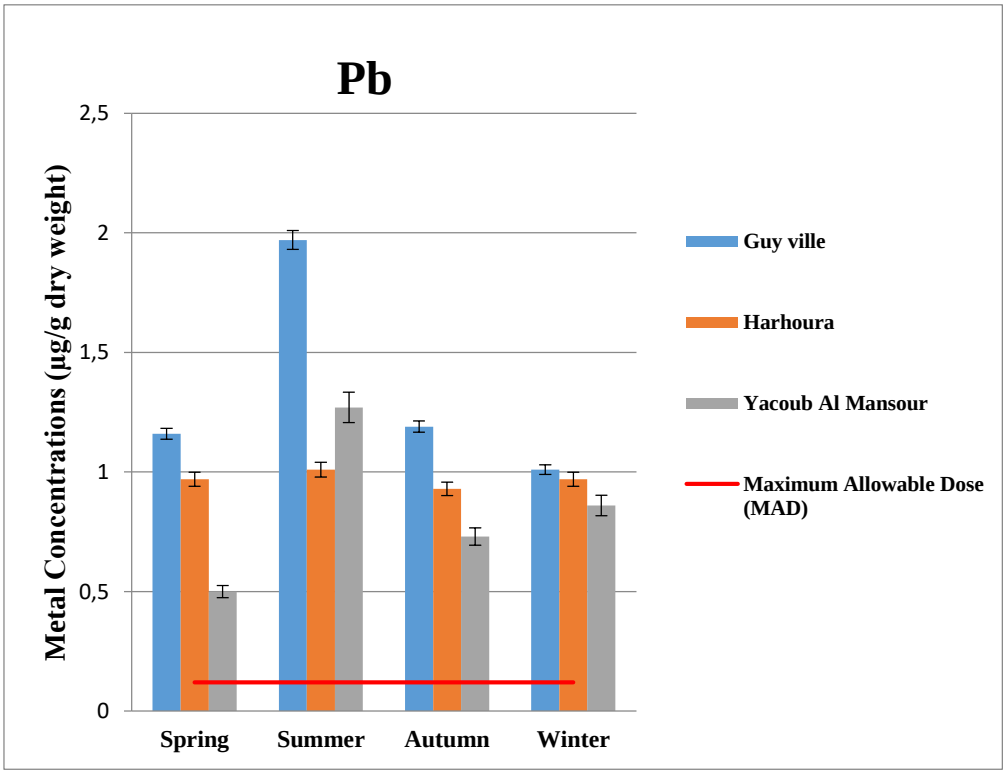


Figure 2. Mean concentrations (µg/g dry weight) of Cu, Cr, Cd and Pb in *Patella rustica* at the different sites during four seasons with Maximum Allowable Dose.

The mean concentrations (in µg/g dry weight) of Copper (Cu), Chromium (Cr), Cadmium (Cd) and Lead (Pb) of the marine gastropod *Patella rustica* at the different sampling sites are summarized in Table 2 during four seasons. The comparison between metal elements concentrations in the samples from the three stations showed all metal concentrations differed significantly in every season (Table 2 and Fig. 2).

The ranges of elements concentrations measured (in µg/g dry weight) found in different stations and seasons were: Cu (1,71±0,07 µg/g), Cr (1,48±0,05 µg/g), Cd (0,72±0,03 µg/g) and Pb (1,05±0,04 µg/g).

The seasonal changes for all metal concentrations in the tissues of Patella rustica from sampling stations were observed in this work. Moreover, the high concentrations of Cu, Cr, Cd and Pb were detected in summer and spring (Table 2 and Fig. 2). Generally high chemical pollution occurred during the summer and spring months (Duysak and Ersoy, 2014; Duysak and Azdural, 2017).

As shown in the Table 2 and Fig. 2 the levels of Cu and Cr have been found to be highest in all tissues in every season while Cd and Pb have been found at lower levels. Cu and Cr are essential elements and play important roles in growth, cell metabolism, and survival of most animals including gastropod molluscs. Therefore, the relatively high levels of these metals can be attributed to their essentiality (Mitra et al., 2012). Other metals have no useful role in the human physiology. Examples of such elements are Cadmium, Lead, Arsenic and mercury (Tchounwou et al., 2012; Jaishankar et al., 2014). The results obtained with the Student t test showed a significant ($p < 0.05$) variation of the Cu, Cr, Cd and Pb concentrations in *Patella rustica* during the four seasons.

Heavy metals may be in certain concentrations, toxic to organisms even at low levels of exposure and persistent in the aquatic environment. Also, can cause diseases, cancers, unfavourably affect their consumption by nutrition, have harmful reproductive effects and displace the biologically useful metals (such as zinc and calcium) (Singh et al., 2011; Tchounwou et al., 2012; Jaishankar et al., 2014). The recommended Maximum Allowable Dose (MAD) of metals concentration in edible gastropod molluscs (such as *Patella)* as follow (3,28 µg/g for Cu; 0,73 µg/g for Cr; 0,19 µg/g for Cd and 0,12 µg/g for Pb) (IAEA-407, 2003-09-01).

The copper (Cu) surplus has been associated with liver damage (Collado et al., 2006). The chromium (Cr) is considered as a human mutagen and probably carcinogen. Prolonged exposure to Cr may cause damage to the liver and kidneys (Velma et al., 2009). The cadmium (Cd) is known to be toxic even at low concentrations and is also considered as a probably carcinogen; Cd can also affect in bone fracture, arthritis, diabetes, anaemia, hypertension, cardiovascular disease, cirrhosis, reduced fertility, headaches, strokes, kidney dysfunction and even cancer (Cravo et al., 2005; Collado et al., 2006). The lead (Pb) is known to be mutagenic and carcinogenic. It induces renal tumours and disturbs the normal functioning of joints, nervous and reproductive systems (Cravo et al., 2005; Collado et al., 2006).

In this study, we report the concentrations of Cu, Cr, Cd and Pb in the Patellidae tissues from three different stations. The results obtained with the Mann-Whitney U test clearly demonstrate a significant ($p < 0.01$) spatial

variations of the Cu, Cr, Cd and Pb concentrations. The highest metal levels were registered in Guy ville coast, followed by Harhoura coast and at last Yacoub Al Mansour coast (Table 3 and Fig. 3).

Table 3. Mean concentrations ± relative standard deviation values (µg/g of dry weight) of metals in *Patella rustica* from different sites.

Elements	Stations		
	Yacoub Al Mansour Coast	Harhoura Coast	Guy ville Coast
Cu	1,13± 0,05	1,67± 0,05	2,32± 0,11
Cr	1,16±0,04	1,42±0,03	1,86±0,08
Cd	0,51±0,02	0,77±0,03	0,89±0,04
Pb	0,84±0,02	0,97±0,04	1,33± 0,06

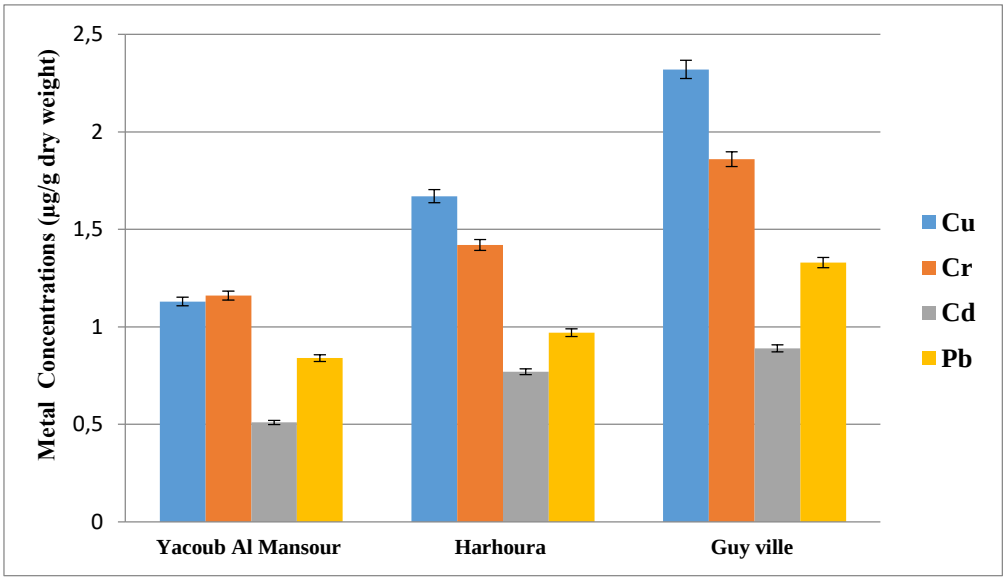


Figure 3. Mean concentrations (µg/g dry weight) of metal elements in *Patella rustica* at the different sites.

The limpet *Patella rustica* is filter feeding organism. Therefore, their ability to accumulate metal elements in their tissues is well known. This accumulation responding essentially to the fraction of metal present in the aquatic environment gives direct indication of the ecotoxicological level of the pollution.

The comparison of metal concentrations with corresponding values measured in species of *Patella* sp. from various geographical sites is shown in Table 4. Such a comparison will additionally provide more useful information on the contamination degree of marine ecosystems. The Cu, Cd and Pb concentrations from Yacoub Al Mansour, Harhoura and Guy ville (Moroccan

Atlantic coast) are quite similar to those obtained by Collado et al. 2006, Bergasa et al., 2007 and Yuzereroglu et al., 2010 from polluted areas, located at Canary Islands (Spain) and Iskenderun Gulf (Turkey). The concentration of copper and chromium fall within the highest available data values in the literature.

Table 4. Comparison of metals concentration (µg/g of dry weight) for *Patella* sp. obtained in this study and from different other geographical areas.

Species	Sites	Mean concentrations ± RSD (µg/g dry weight)				References
		Cu	Cr	Cd	Pb	
Patella rustica	Yacoub Al Mansour coast (Morocco)	1,13± 0,05	1,16±0,04	0,51±0,02	0,84±0,02	This work
	Harhoura coast (Morocco)	1,67± 0,05	1,42±0,03	0,77±0,03	0,97±0,04	
	Guy Ville coast (Morocco)	2,32± 0,11	1,86±0,08	0,89±0,04	1,33± 0,06	
Patella rustica	Canary Islands (Spain)	2.05 ± 0.91	-	0.36 ± 0.26	1.57 ± 1.14	Bergasa et al., 2007
Patella piperata		1.77 ± 0.09	-	0.37 ± 0.05	1.27 ± 0.07	Collado et al., 2006
Patella caerulea	Gulf of Annaba (Algeria)	4,56±0,69	15,88±0,59	-	-	Boumaza, 2014
	Iskenderun Gulf (Turkey)	2,31±0,09	-	0,44±0,03	0,30±0,03	Yuzereroglu et al., 2010
	Gulf of Suez (Egypt)	6,34±1,96	4,68±1,43	1,38±1,12	6,2±1,75	Hamed and Emara 2006
	Gulf of Gaeta, Tyrrhenian Sea (Italy)	14,3±3,43	0,85±0,23	3,54±0,78	0,95±0,20	Conti and Cecchetti 2003
Patella vulgata	Lebanese Coastal (Lebanon)	-	-	2,16±0,26	1,55±0,34	Nakhle et al., 2006
Patella aspera	South coast (Portugal)	6,3±1,72	-	3,5±0,57	-	Cravo and Bebianno 2005

In this work, the presence of high Cu, Cr, Pb and Cd concentrations in limpet samples from (Yacoub Al Mansour, Harhoura and Guy ville) coast should be attributed to domestic discharges, the waste incineration and the exhaust gas of the vehicles. All these factors may have affected the *Patella rustica*, which indicate the susceptibility of this specie to the chemical pollution in the study area (Moroccan Atlantic Coast).

The metal concentrations in limpet depend on both the species and the physiological state of the organism (Nakhle, 2003). Indeed, the real mechanism of metals in *Patella* was complicated and depends on factors directly correlated with weight, sexual cycle, temperature, food abundance and the bioavailability of metals in the environment (Nakhle, 2003; Storelli et al., 2005; Bergasa, 2009).

Conclusion

Patellidae is commonly consumed seafood in many countries. Therefore, the investigation of metal elements concentrations in the tissues of this species may provide useful information on the transfer of potentially toxic elements from aquatic environment. The usefulness of gastropod molluscs as bio-indicators for the detection of metal pollution is confirmed. However, the effect of some variables on metal concentrations in gastropod molluscs may be different, according to the sampling period and to the level of metal pollution of the area under investigation.

The results of this study conclude that *Patella rustica* are seriously polluted by metal elements, especially from Cu, Cr, Pb and Cd. The most important source of pollution in these sampling sites was the anthropogenic activities and the road traffic.

The data obtained from this study revealed the necessity to establish a monitoring system to control the consumption of *Patella rustica* from the Moroccan Atlantic coast and to evaluate the toxicological risk related to the contamination of this species.

Patella has a considerable potential as cosmopolitan bio-monitors of metals in the aquatic environment. This sedentary species are available in every season all over the coastal area and is easy to sample and identify. Finally, it is recommended that *Patella rustica* are suitable to be used as a successful bio-indicator of metal pollution caused by different human activities in this coastal area of the Moroccan Atlantic coast and should be controlled before their consumption.

Further studies of metal levels in gastropod molluscs from different sampling sites of the Moroccan Atlantic coast are required including the investigation of the possible effects of seasonal changes on metal concentrations and distribution.

Acknowledgements

This work was supported by the laboratory of toxicology in National Institute of Hygiene (Rabat, Morocco). We want to thank the team of this laboratory for his technical assistance.

Disclosure statement

No potential conflict of interest was reported by the authors.

Notes on contributors

Dr. Lalla Meryem IDRISSI AZZOUZI - Laboratory of Virology, National Institute of Hygiene in Rabat and Laboratory of Biodiversity, Ecology and Genome, University Mohamed V of Rabat, School of Sciences, MOROCCO

Dr. Rachid Ben Aakame - Laboratory of Hydrobiology, Toxicology, Industrial and Environmental Hygiene, National Institute of Hygiene, Rabat, MOROCCO

Dr. Abdellah El Abidi - Laboratory of Hydrobiology, Toxicology, Industrial and Environmental Hygiene, National Institute of Hygiene, Rabat, MOROCCO

Prof. Mariam Naciri - Laboratory of Biodiversity, Ecology and Genome, University Mohamed V of Rabat, School of Sciences, MOROCCO

References

Baby, J., Raj, J. S., Biby, E. T., Sankarganesh, P., Jeevitha, M.V., Ajisha, S.U., Rajan, S. S. (2010). Toxic effect of heavy metals on aquatic environment. *Int. J. Biol. Chem. Sci*, 4(4), 939-952.

Bergasa, O., Ramirez, R., Collado, C., Hernandez-Brito, J. J., Gelado-Cabarello, M. D., Rodriguez-Somozas, M., Haroun, R. J. (2007). Study of metals concentration levels in *Patella piperata* throughout the Canary Islands, Spain. *Environ Monit Assess*, 127, 127-133.

Ben Aakame, R., Fekhaoui, M., El Abidi, A., Dussauze, J., Laghzizal, M., Saoiabi, A. (2014). Groundwater contamination by pesticides and metals elements in agricultural areas of the Northwest of Morocco and health hazard. *IOSR-JESTFT*, 8(1), 68-71.

Bergasa, O. (2009). Study of metals concentrations levels in *Patella piperata* through the Canary Islands, Spain. *Fresenius Environmental Bulletin*, 15, 1234-1240.

Boumaza, F., Z. (2014). Évaluation de l'état de santé des eaux du golfe d'Annaba à travers un Mollusque Gastéropode *Patella caerulea* (L., 1758): paramètres écologiques et biochimiques. *These*, 1-194.

Bradl, H. (2005). Heavy Metals in the Environment: Origin, Interaction and Remediation. *Interface science and Technology*, 1(6), 282.

Chaibi, M., & Sedrati, M. (2009). Coastal erosion induced by human activities: the case of two embayed beaches on the Moroccan coast. *Journal of Coastal Research*, SI56, 1184-1188.

Collado, C., Ramirez, R., Bergasa, O., Hernandez-Brito, J. J., Gelado-Caballero, M. D., Haroun, R., J. (2006). Heavy metals (Cd, Cu, Pb and Zn) in two species of limpets (*Patella rustica* and *Patella candei crenata*) in the Canary Islands, Spain. *WIT Transactions on Ecology and the Environment*, 95, 45-53.

Conti, M. E., Cecchetti, G. (2003). A biomonitoring study: trace metals in algae and molluscs from Tyrrhenian coastal areas. *Environ Res*, 93, 99-112.

Cravo, A., Bebianno, M., J. (2005). Bioaccumulation of metals in the soft tissues of *Patella aspera*: Application of metal/shell weight indices. *Estuar Coast Shelf Sci*, 65, 571-586.

Duysak, O., and Ersoy, B. (2014). A biomonitoring study: heavy metals in Monodonta turbinata (Mollusca: Gastropoda) From Iskenderun Bay, North-Eastern Mediterranean. *Pakistan J. Zool*, 46, 1317-1322.

Duysak, O., and Azdural, K. (2017). Evaluation of Heavy Metal and Aluminium Accumulation in a Gastropod, Patella caerulea L., 1758 in Iskenderun Bay, Turkey. *Pakistan J. Zool*, 49(2), 629-637.

Glasby, G. P., Szefer, P., Geldon, J., Warzocha, J. (2004). Heavy-metal pollution of sediments from Szczecin Lagoon and the Gdansk Basin, Poland. *Science of the Total Environment*, 330, 249–269.

Hamed, M. A., Emara, A. M. (2006). Marine molluscs as biomonitors for heavy metals in the Gulf of Suez, Red Sea. *J Mar Syst*, 60, 220-234.

He, Z. L., Yang, X. E., Stoffella, P. J. (2005). Trace elements in agroecosystems and impacts on the environment. *J Trace Elem Med Biol*, 19(2–3)125–140.

Idrissi Azzouzi, L. M., Senouci, S., El Qazoui, M., Oumzil, H., Naciri, M. (2017). Detection of enterovirus in mussels from Morocco by cell culture and real-time PCR. *Afr. J. Biotechnol.* 16(34), 1791-1799.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdiscip Toxicol.* 7(2), 60-72.

Kelepertzis, E. (2013). Heavy Metals Baseline Concentrations In Soft Tissues Of *Patella* Sp. From The Stratoni Coastal Environment, Ne Greece. *ECOL CHEM ENG S*, 20(1), 141-149.

Maanan, M., Zourarah, B., Carruesco, C., Aajjane, A., Naud, J. (2004). The distribution of heavy metals in the Sidi Moussa lagoon sediments (Atlantic Moroccan Coast). *Journal of African Earth Sciences*, 39, 473-483.

Maanan, M. (2008). Heavy metal concentrations in marine molluscs from the Moroccan coastal region. *Environmental Pollution*, 153, 176-183.

Maanan, M., Zourarah, B., Sahabi, M., Maanan, M., Le Roy, P., Mehdi, K., Salhi, F. (2015). Environmental risk assessment of the Moroccan Atlantic continental shelf: The role of the industrial and urban area. *Science of the Total Environment*. 511, 407-415.

Mitra, A., Barua, P., Zaman, S., Banerjee, K. (2012). Analysis of Trace Metals in Commercially Important Crustaceans Collected from UNESCO Protected World Heritage Site of Indian Sundarbans. *Turk. J. Fish. Aquat. Sci.* 12, 53-66.

Mulgrave, V. (2017). Flame Atomic Absorption Spectrometry Analytical Methods. *Agilent Technologies*. 14, 23-41.

Nakhle, K. F., Cossa, D., Khalaf, G., Beliaeff, B. (2006). *Brachidontes variabilis* and *Patella* sp. as quantitative biological indicators for cadmium, lead and mercury in the Lebanese coastal waters. *Environmental Pollution*, 142, 73-82.

Richir and Gobert. (2016). Trace Elements in Marine Environments: Occurrence, Threats and Monitoring with Special Focus on the Coastal Mediterranean. *J Environ Anal Toxicol*, 6(1), 1-19.

Sa-Pinto, A., Baird, S. J. E., Pinho, C., Alexandrino, P., Branco, M. (2010). A three-way contact zone between forms of Patella rustica (Mollusca: Patellidae) in the central Mediterranean Sea. *Biological Journal of the Linnean Society*, 100, 154-169.

Singh, R., Gautam, N., Mishra, A., Gupta, R. (2011). Heavy metals and living systems: An overview. *Indian J. Pharmacol*, 43(3), 246–253.

Singh, N.K. S., Sudarshan, M., Chakraborty, A., Devi, C. B., Singh, T. B., Singh, N. R., (2014). Biomonitoring of Fresh Water of Loktak Lake, India. *European Journal of Sustainable Development*, 3(1), 179-188.

Snoussi, M., Ouchani, T., Niazi, S. (2008). Vulnerability assessment of the impact of sea-level rise and flooding on the Moroccan coast: The case of the Mediterranean eastern zone. *Estuarine Coastal and Shelf Science*, 77, 206-213

Storelli, M. M., & Marcotrigiano, G., O. (2005). Bioindicator organisms: Heavy metal pollution evaluation in the Ionian Sea (Mediterranean sea-Italy). *Environmental Monitoring and Assessment*, 102, 159-166.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., Sutton, D. J. (2012). Heavy Metals Toxicity and the Environment. *NIH Public Access*, 101, 133-164.

Velma, V., Vutukuru, S. S., Tchounwou, P. B. (2009). Ecotoxicology of Hexavalent Chromium in Freshwater Fish: A Critical Review. *Rev Environ Health*, 24(2), 129-145.

Yuzereroglu, T. A., Gok, G., Cogun, H. Y., Firat, O., Aslanyavrusu, S., Maruldali, O., Kargin, F. (2010). Heavy metals in *Patella caerulea* (mollusca, gastropoda) in polluted and non-polluted areas from the Iskenderun Gulf (Mediterranean Turkey). *Environ Monit Assess*, 167(1-4), 257-264.

Wani, R. A., Ganai, B. A., Shah, M. A., Uqab, B. (2017). Heavy Metal Uptake Potential of Aquatic Plants through Phytoremediation Technique - A Review. *J. Bioremediat Biodegrad*, 8(4), 1-5.