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Full Length Research Paper

The relationships between metal (Pb, Cu, Cd and Cr) levels and the size of the Moroccan Atlantic Coast gastropod species (*Patella rustica*)

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The metal (Pb, Cu, Cd, and Cr) concentrations in the soft tissues of the gastropod mollusc limpet (*Patella rustica*) from the Moroccan Atlantic Coast were measured. The relationships between limpets size and metal levels were investigated by linear regression analysis. This work was carried out to determine the bioaccumulation and relationships of some essential (Cu and Cr) and non-essential (Pb and Cd) metals in the soft tissues of *P. rustica* by calculating the correlation analysis. For this study, 120 limpets (*P. rustica*) were collected from three different locations in the purpose to analyse the levels of metals by using the standard Atomic Absorption Spectrophotometry (AAS). In soft tissues of *P. rustica*, the metal concentrations (ng/mg dry weight) decrease in the following order: Cu (4.14) > Cr (3.98) > Pb (2.13) > Cd (1.18). The results of the linear regression analysis confirmed that in all samples the relationships between metal concentrations and limpets size were significant. Moreover, the smaller size of limpets showed higher concentrations (ng/mg dry weight) of Cd, Pb, Cu and Cr than the larger ones. However, it was found that the tissue of *P. rustica* has the potential to be used as a biomonitoring agent for the metal contamination in gastropod molluscs, as indicated by the significant correlation between metal concentrations (Pb, Cu, Cd, and Cr) in the soft tissues of limpets and their size.

Key words: Metals, *Patella rustica*, length, weight, bioaccumulation, Moroccan Atlantic Coast.

INTRODUCTION

The expression "heavy metals" is generally used as a group name for metals that have very toxic properties. They are considered as serious contaminants in the environment, due to their high potential to enter and

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accumulate in food chains (Duffus, 2002; Begum and Sehrin, 2013; Kouddane et al., 2016; Hazrat et al., 2019). By cause of their great degree of toxicity, arsenic, cadmium, chromium, lead, and mercury are among the priority metals important for public health (Türkmen and Ciminli, 2006; Boyd et al., 2010; Jakimska et al., 2011; Tchounwou et al., 2012; Ayangbenro and Babalola, 2017).

The gastropod mollusc, *Patella rustica* was chosen because they are often considered as a keystone species on rocky shores and several studies have demonstrated that limpet has the ability to accumulate metals in their soft tissues responding essentially to the fraction present in the environment, which is of direct ecotoxicological relevance. Therefore, it is considered as a bioindicator of metal contaminations in the aquatic ecosystems (Davies and Hatcher, 1999; Lopez et al., 2003; Battelli, 2016; Jellison et al., 2016). These gastropods are commonly harvested and consumed by humans around the world. It could possibly be a harmful metal transfer to the food chains (Wang, 2002; Collado et al., 2006; Bergasa et al., 2007; Vinas et al., 2018; Türk-Çulha et al., 2022).

The metal concentrations measured in the tissue of molluscs could be used as biomonitors of metal bioavailability and contamination in the coastal marine environment, in which they live. However, the accumulation of metals in the molluscs also get affected by a number of intrinsic such as environmental stress and extrinsic factors such as spawning season and body size (Davies et al., 2005; Türkmen et al., 2005; Yap et al., 2009; Sarkar et al., 2008; Azizi et al., 2018; Türk-Çulha et al., 2022). Previous work has revealed that the body size may alter the absorption of heavy metal due to changes in environmental conditions. Evidently, the size of the organism would affect the bioaccumulation of metals in absorption and excretion rates. Moreover, the effects of the body size on different physiological levels such as filtration and respiration have been reported in molluscs (Wang, 2002; Jakimska et al., 2011). Until now, no study has identified the relationships between metal levels and the size of the Moroccan Atlantic Coast gastropod species such as *P. rustica*. Therefore, the present study aimed to offer comparative information in understanding the physiological strategies for the accumulation of Pb, Cu, Cd, and Cr in relation to limpets size. Consequently, the objective of this work was to investigate the effects of the length and weight on metal concentrations (ng/mg dry weight) in the soft tissues of *P. rustica*.

MATERIALS AND METHODS

Study area

The sampling locations were situated in Rabat Region of Morocco (Figure 1). Covering this region an area of 18.194 km^2, with a population of about 4.581.000. This area belongs to the Mediterranean climate characterized by two main seasons softened by oceanic influences. The average temperatures are approximately 22°C for the warmer months (July to September) and 12°C for the colder months (December and January). Relating to the annual rainfall is in average more than 550 mm/year (Idrissi Azzouzi et al., 2017a, b).

Samples collection

Among February 2015 to February 2016, a total of 120 limpets (*P. rustica*) with approximate size (0.24-3.59 g tissue dry weight) were collected from three wild populations (Yacoub Al Mansour, Harhoura and Guy Ville Coast) of Moroccan Atlantic Coast in Rabat-Sale-Kenitra region. These sites receive large quantities of untreated or partially treated domestic wastewater.

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

Samples preparation, analysis and data analysis

In the present study, the breadth, length and height of the shell (Figure 2) was measured using a vernier caliper giving a measurement of 1/10th of a millimeter; the soft tissues of samples were weighed after their preparation using a precision balance. The sample preparation and the analysis of the metal concentrations in the soft tissues of limpet were described by Idrissi Azzouzi et al. (2017b).

The data analysis was carried out by means of the statistical package, R version 3.4.1 software. The correlation test was used to check for significant relationships between metal concentrations and limpets size. The level of significance was set at a probability lower than 0.05 ($p < 0.05$). To evaluate significant differences between groups, the Levene test was applied to verify the equality of variances. Subsequently, ANOVA or Kruskal-Wallis test were applied according to the distribution of the data (normal or not, respectively).

RESULTS AND DISCUSSION

All biometric relationships performed on *P. rustica* collected showed a significant correlation. These correlations indicate that the limpets of the Moroccan Atlantic Coast are in biological balance. Therefore, the more the coefficient of determination R^2 gets close to 1, the correlation is better and the individuals are in biological balance (Figure 3). On the set of the individuals, we find a class of Shell length between 20 and 44 mm and tissue dry weight between 239 and 3590 mg (Table 1).

The logarithmic relationships between shell length and dry weight of the limpet tissues were determined by using parabolic form of the following equation (W= aLn, where W= Weight (in mg), L= Length (in mm), a is a constant and n an exponent usually between 2.5 and 4.0), this correlation indicates an allometric growth, that is, the length becomes an irrelevant variable in relation to the weight (Figure 3a).

Figure 1. Location of the sampling sites (Yacoub Al Mansour, Harhoura and Guy Ville Coast).

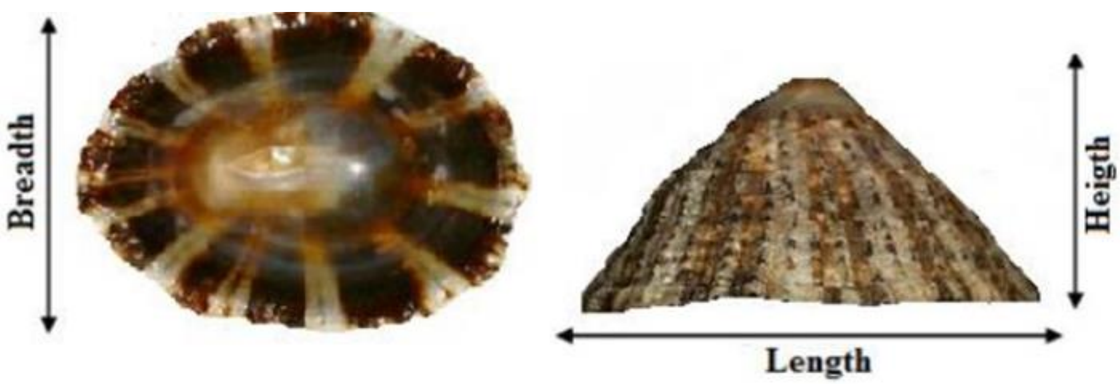

Figure 2. Shell dimensions of *Patella rustica*: breadth, length and heigth.

The correlation between shell length and shell weight of the limpet was described using a power regression equation (Figure 3b). Whereas the relation between shell weight and dry weight of limpet tissues is linear (Figure 3c); this assumes that, although the growth of the shell length is slower, its weight remains increasing. The relationships between the shell length on one hand, its breadth and height on the other hand is linear and highly significant (Figure 3d and e). This means that the shell of limpet (*P. rustica*) has a conical shape, so its growth in height is the result of an increase in its base (the length-breadth is large or small depending on the diameter of shell).

In this study, the results of the linear regression analysis showed that the relationships between metal concentrations and limpets size were significant. Highly significant negative relationships ($p < 0.001$) were found between shell length and concentrations of lead (Pb) and copper (Cu) in the soft tissues of *P. rustica* (Figure 4a and b). The shell length and concentrations of cadmium (Cd) and chromium (Cr) showed significant positive relationships ($p < 0.05$) (Figure 4c and d).

The results of this study demonstrated that the plotting of the metal content, versus tissue dry weight, gave good straight lines; this indicates that *P. rustica* presented a different physiological strategy for each metal studied, which is related to the size of limpets. These explain the presence of a significant correlation between

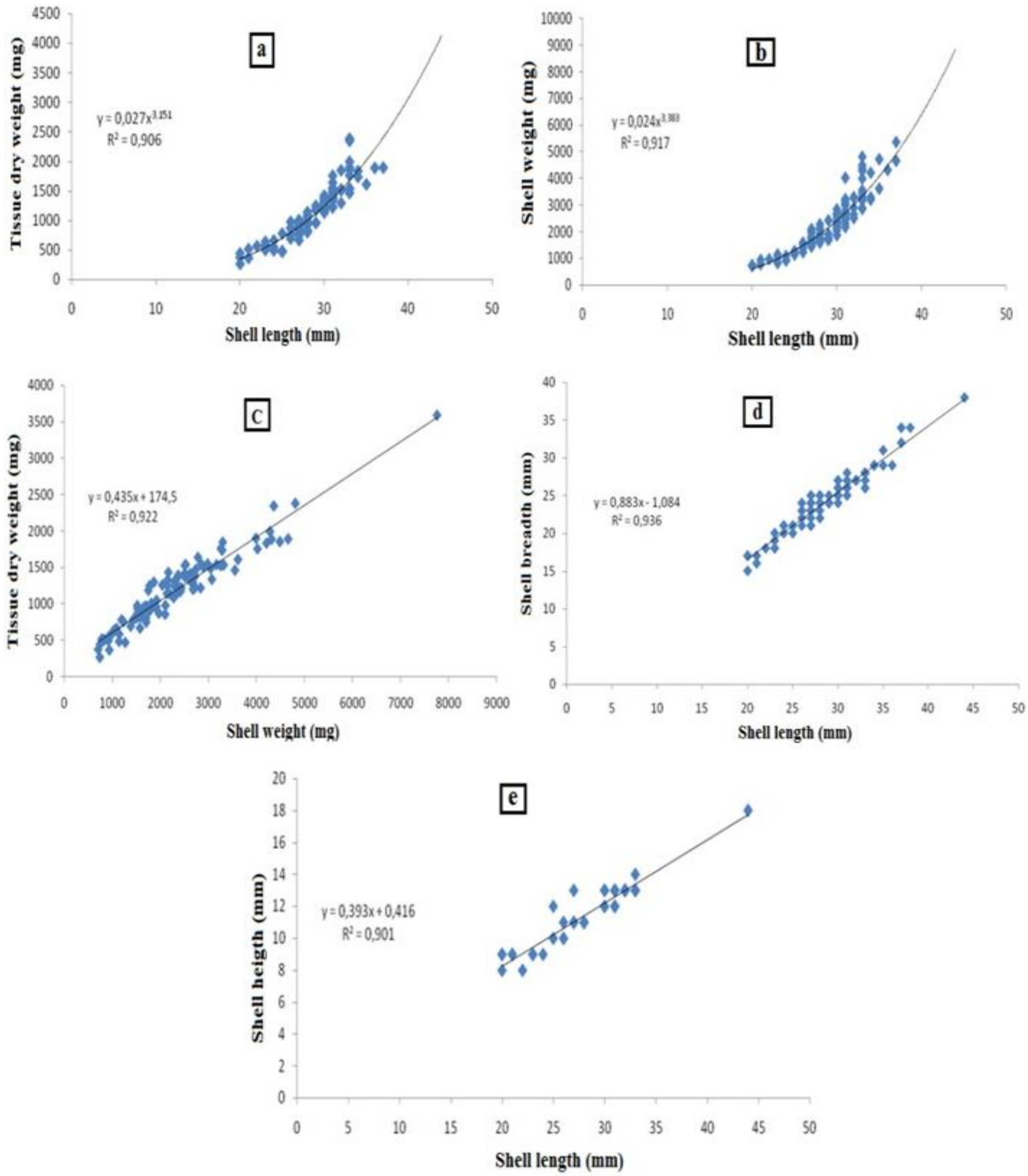

Figure 3. Relationships between all size components of *Patella rustica*.

Table 1. Sampling sites of limpet (*Patella rustica*) and their sizes.

Site	Shell length (mm) (Min-Max)	Shell breath (mm) (Min-Max)	Shell height (mm) (Min-Max)	Shell weight (mg) (Min-Max)	Tissue dry weight of limpet (mg) (Min-Max)
Yacoub Al Mansour Coast	24-35	18-34	8-16	298-5779	239-2343
Harhoura Coast	21-38	15-35	8-15	266-6216	271-2126
Guy Ville Coast	20-44	18-38	8-18	741-7760	263-3590

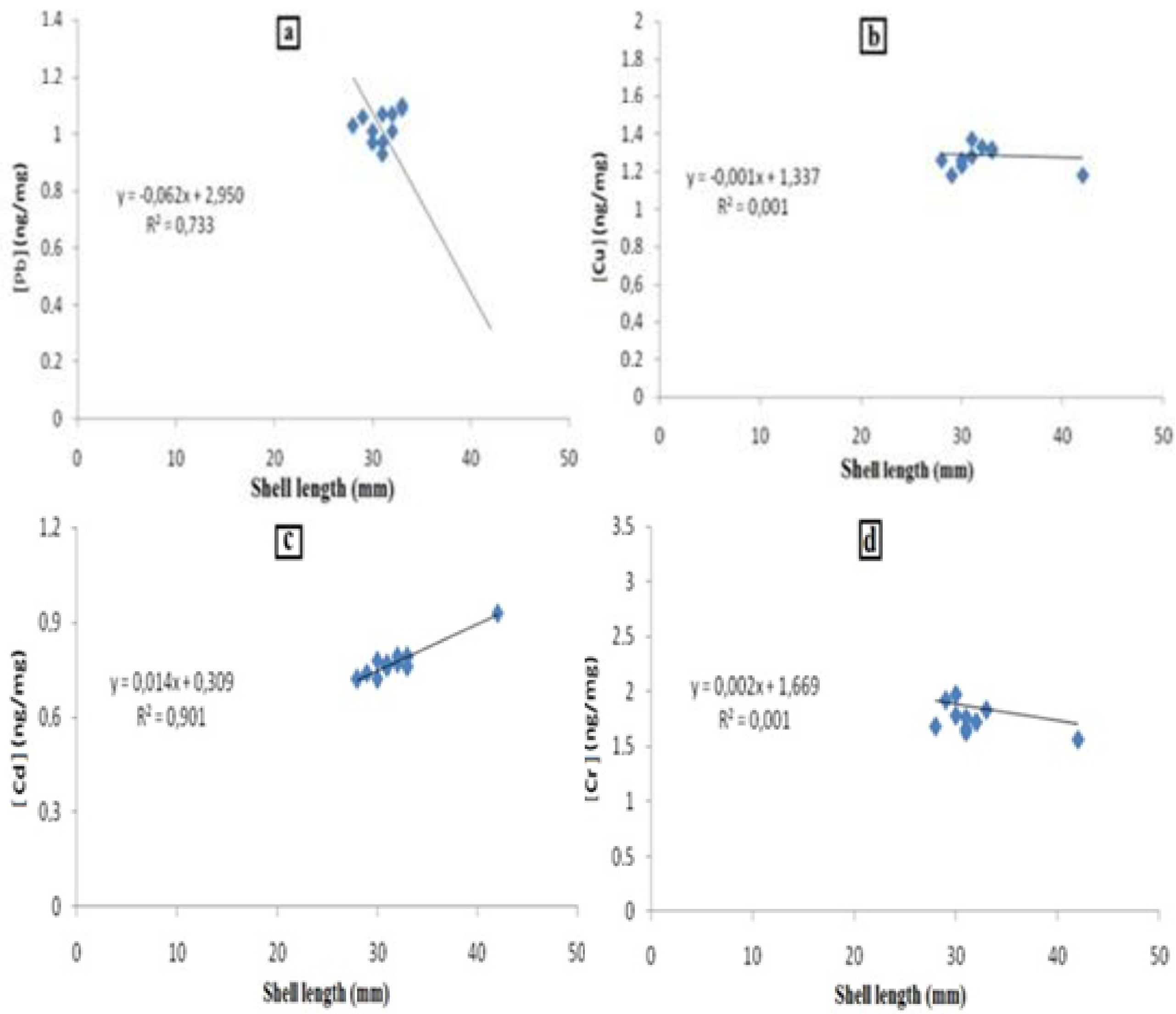

Figure 4. Correlation between shell length and metal (Pb, Cu, Cd, and Cr) concentrations in *Patella rustica*.

bioaccumulation of metals and physiological indices (Figure 4).

Growth parameters (length, weight and condition factor) showed a significant relationships with metal concentrations (Pb, Cu, Cd, and Cr) in the soft tissue of samples. Also, the smaller limpets showed higher concentrations (ng/mg dry weight) of Pb, Cu, Cd, and Cr than the larger ones at each site (Figure 5).

The condition factor (CF) based on the length-weight relationships is often used to express the overall wellbeing of molluscs, this parameter being affected by habitat quality and food availability, thus it can be calculated according to the following formula: CF=W/Lb ×100, where W= Weight, L= Length. The exponent b is derived from the length-weight relationships (Bervoets and Blust, 2003; Banerjee et al., 2016).

The metal (Pb, Cu, Cd, and Cr) concentrations in the tissue of limpets studied at different sites show great variations, with the highest concentrations of Cu, Cd and Cr existing in Guy Ville Coast station. While the concentration of Pb is very important in Harhoura Coast station (Figure 5).

Previous studies showed that at the level of metal accumulations by the bioindicator, there are two groups of metals (Bervoets and Blust, 2003; Canli and Atli, 2003; Nakhle, 2003; Yi and Zhang, 2012; Banerjee et al., 2016): Cu and Pb, these two metals are influenced by the size of the individual; Cd and Cu, these two elements

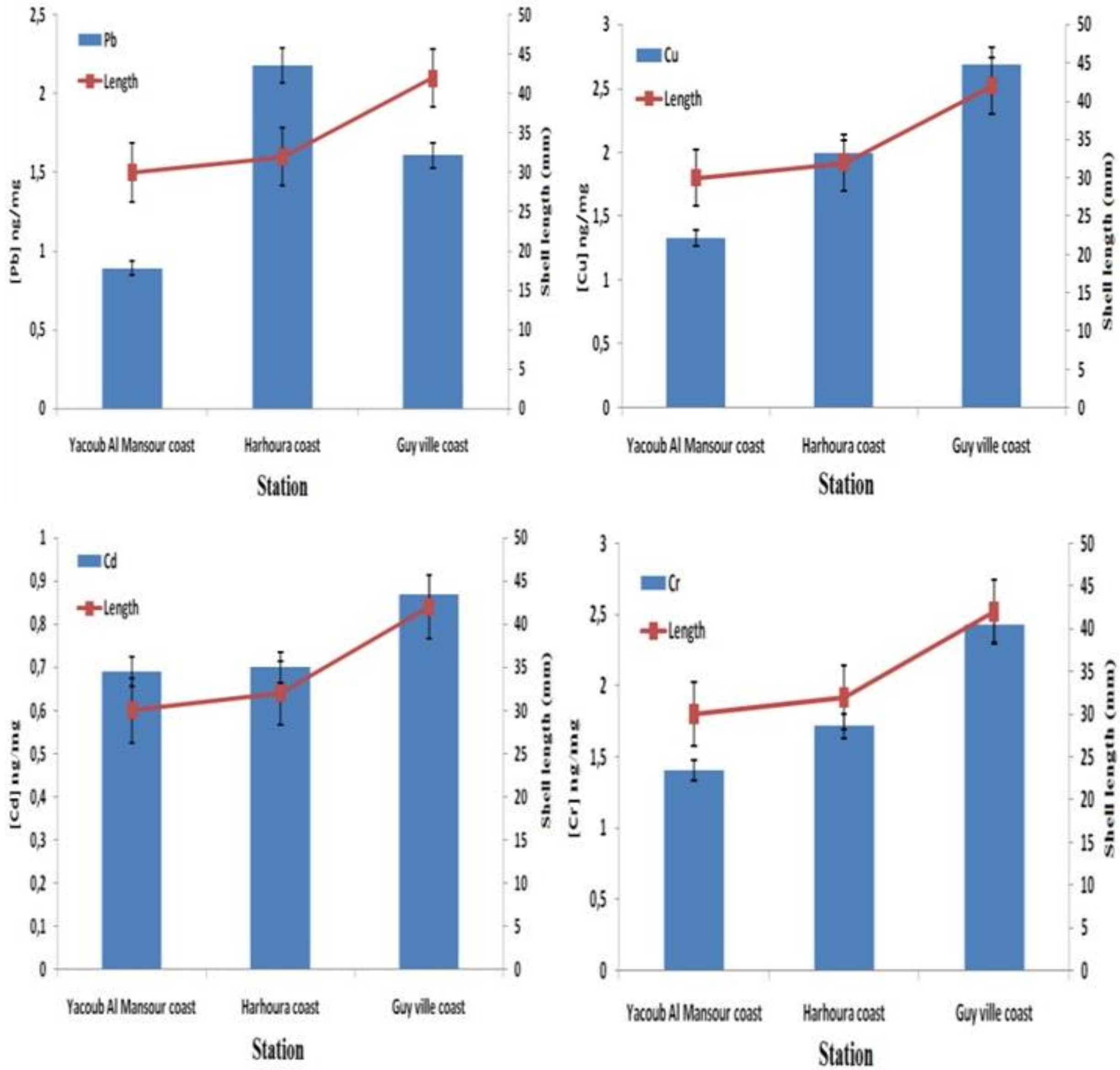

Figure 5. Correlation between concentrations of (Pb, Cu, Cd, and Cr) and shell length in three sites.

correlated with the level of environmental contamination especially for large size of individual.

Other works showed a considerable difference during all seasons, reproductive cycle and in the different geographical position (Jenkins and Hartnoll, 2001; Türkmen and Türkmen, 2005; Monsefrad et al., 2012; Banerjee et al., 2016; Türk-Çulha et al., 2022).

The studies of Cubadda et al. (2001) and Nakhle (2003) showed that there is a power-type relationships between the concentration of the metal and the total weight of the individual. The authors suggest that *P. rustica* functions as a bioindicator of marine pollution but also assume that it has a very high coefficient of concentration of Cd. They linked this fact to the nutritional habits and to the morphological and physiological effects of the species. This study was mainly aimed to investigate relationships between metal concentrations in the soft tissues of *P. rustica* and the size of these limpets (generally the length and the weight).

The statistical analysis revealed that metal concentrations in limpet depend on the type of the species and the physiological condition of the organism (Nakhle, 2003; Yap et al., 2009; Idrissi Azzouzi et al., 2017b). Certainly, the real mechanism of metals bioaccumulation in *P. rustica* is associated with factors that directly correlate with length, weight, age, sexual cycle, temperature, and food abundance (Nakhle, 2003; Storelli and Marcotrigiano, 2005; Bergasa, 2009; Idrissi Azzouzi et al., 2017b).

The results obtained from this study indicate that the concentration of metals (Pb, Cu, Cd, and Cr) in *Patella* varies significantly depending on the size of the limpets and the pollution load of each site (Lopez et al., 2003; Nakhle, 2003; Collado et al., 2006; Bergasa et al., 2007). High concentrations of Cd in the soft tissues of limpets may be the result of a combination of several factors: the behaviour of the animal in its search for food and the adsorption of metals on the mucus. These metals especially Pb, Cu, Cd, and Cr adsorb on the mucus deposited by the limpets during its dislocation (Lopez et al., 2003; Nakhle, 2003; Collado et al., 2006; Bergasa et al., 2007).

This mucus fixed on the bedrock and on the bacterial and algal film with high metal concentrations even in non-contaminated environments. The displacement of the limpet on the same location or trail obligates him to feed on microalgae already impregnated with this mucus. The concentration of metals is increased in the new mucus secreted. Therefore, there is an increase in concentration of metals even if the sites are slightly contaminated or uncontaminated (Lopez et al., 2003; Nakhle, 2003; Collado et al., 2006; Bergasa et al., 2007; Ayangbenro and Babalola, 2017).

Conclusion

The present study revealed that smaller size of *P. rustica* accumulated higher concentrations of Pb, Cd, Cu, and Cr; as well as the relationships between the accumulation of metal and size were confirmed. Therefore, the factor of body size of samples should be considered in all studies. Based on both the characteristics of gastropod molluscs (bioaccumulators, biomonitors and bioindicators) and the quality of the marine environment, the levels of Pb, Cd, Cu, and Cr observed in the tissue of limpets studied for the three sites (Yacoub Al Mansour, Harhoura and Guy Ville Coast) should be considered to be an important warning signal.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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