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

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**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 Guyville) 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: 0.94-2.3 μg/g; Cd: 0.47-0.95 μg/g and Pb: 0.76-1.42 μg/g) and Guyville 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**


**ARTICLE HISTORY**

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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...
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 (Yuzeroglu et al., 2016; 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](image)

**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 (HNO₃) 65% (Merck) and 1 mL of oxygenated water (H₂O₂) 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 (mean± relative standard deviation) (in µg/g of dry weight).
**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 Guyville 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.
<table>
<thead>
<tr>
<th>Elements</th>
<th>Stations</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>Yacoub Al Mansour coast</td>
<td>1,18±0,05</td>
<td>1,08±0,03</td>
<td>1,06±0,05</td>
<td>1,18±0,06</td>
</tr>
<tr>
<td></td>
<td>Harhoura coast</td>
<td>1,47±0,02</td>
<td>2,92±0,14</td>
<td>1,01±0,03</td>
<td>1,26±0,02</td>
</tr>
<tr>
<td></td>
<td>Guy ville coast</td>
<td>1,41±0,01</td>
<td>5,51±0,45</td>
<td>1,41±0,04</td>
<td>0,93±0,06</td>
</tr>
<tr>
<td>Cr</td>
<td>Yacoub Al Mansour coast</td>
<td>1,63±0,07</td>
<td>1,02±0,02</td>
<td>1,56±0,04</td>
<td>1,47±0,02</td>
</tr>
<tr>
<td></td>
<td>Harhoura coast</td>
<td>1,26±0,06</td>
<td>1,46±0,04</td>
<td>0,88±0,02</td>
<td>1,02±0,03</td>
</tr>
<tr>
<td></td>
<td>Guy ville coast</td>
<td>1,83±0,09</td>
<td>2,96±0,16</td>
<td>1,68±0,06</td>
<td>0,98±0,02</td>
</tr>
<tr>
<td>Cd</td>
<td>Yacoub Al Mansour coast</td>
<td>0,43±0,01</td>
<td>0,90±0,04</td>
<td>0,32±0,01</td>
<td>0,40±0,01</td>
</tr>
<tr>
<td></td>
<td>Harhoura coast</td>
<td>0,87±0,03</td>
<td>0,79±0,02</td>
<td>0,56±0,03</td>
<td>0,87±0,03</td>
</tr>
<tr>
<td></td>
<td>Guy ville coast</td>
<td>0,96±0,07</td>
<td>1,07±0,02</td>
<td>0,60±0,01</td>
<td>0,92±0,04</td>
</tr>
<tr>
<td>Pb</td>
<td>Yacoub Al Mansour coast</td>
<td>0,50±0,01</td>
<td>1,27±0,01</td>
<td>0,73±0,03</td>
<td>0,86±0,02</td>
</tr>
<tr>
<td></td>
<td>Harhoura coast</td>
<td>0,97±0,03</td>
<td>1,01±0,02</td>
<td>0,93±0,04</td>
<td>0,97±0,05</td>
</tr>
<tr>
<td></td>
<td>Guy ville coast</td>
<td>1,16±0,06</td>
<td>1,97±0,08</td>
<td>1,19±0,07</td>
<td>1,01±0,02</td>
</tr>
</tbody>
</table>
The graphs show the metal concentrations (µg/g dry weight) for Cu and Cr, categorized by season (Spring, Summer, Autumn, Winter) for three locations: Guy ville, Harhoura, and Yacoub Al Mansour. The y-axis represents metal concentrations, while the x-axis indicates the seasons.

For Cu:
- Guy ville shows the highest concentration in Summer with a value close to 5 µg/g dry weight.
- Harhoura also has a high concentration in Summer, slightly lower than Guy ville.
- Yacoub Al Mansour has consistently lower concentrations compared to the other two locations across all seasons.

For Cr:
- Guy ville has the highest concentration in Summer, similar to Cu.
- Harhoura again shows a high concentration in Summer, nearly matching Guy ville.
- Yacoub Al Mansour continues to have the lowest concentrations for Cr as well.

A red line on each graph represents the Maximum Allowable Dose (MAD) for each metal.
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.

<table>
<thead>
<tr>
<th>Elements</th>
<th>Stations</th>
<th>Yacoub Al Mansour Coast</th>
<th>Harhoura Coast</th>
<th>Guy ville Coast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td></td>
<td>1.13± 0.05</td>
<td>1.67± 0.05</td>
<td>2.32± 0.11</td>
</tr>
<tr>
<td>Cr</td>
<td></td>
<td>1.16±0.04</td>
<td>1.42±0.03</td>
<td>1.86±0.08</td>
</tr>
<tr>
<td>Cd</td>
<td></td>
<td>0.51±0.02</td>
<td>0.77±0.03</td>
<td>0.89±0.04</td>
</tr>
<tr>
<td>Pb</td>
<td></td>
<td>0.84±0.02</td>
<td>0.97±0.04</td>
<td>1.33± 0.06</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sites</th>
<th>Mean concentrations ± RSD (µg/g dry weight)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Patella rustica</em></td>
<td>Yacoub Al Mansour coast (Morocco)</td>
<td>Cu 1.13± 0.05, Cr 1.16±0.04, Cd 0.51±0.02, Pb 0.84±0.02</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>Harhoura coast (Morocco)</td>
<td>Cu 1.67± 0.05, Cr 1.42±0.03, Cd 0.77±0.03, Pb 0.97±0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Guy Ville coast (Morocco)</td>
<td>Cu 2.32± 0.11, Cr 1.86±0.08, Cd 0.80±0.04, Pb 1.33± 0.06</td>
<td></td>
</tr>
<tr>
<td><em>Patella rustica</em></td>
<td>Canary Islands (Spain)</td>
<td>Cu 2.05 ± 0.91, Cr - 0.36 ± 0.26, Pb 1.57 ± 1.14</td>
<td>Bergasa et al., 2007</td>
</tr>
<tr>
<td><em>Patella piperata</em></td>
<td></td>
<td>Cu 1.77 ± 0.09, Cr - 0.37 ± 0.05, Pb 1.27 ± 0.07</td>
<td>Collado et al., 2006</td>
</tr>
<tr>
<td><em>Patella caerulea</em></td>
<td>Gulf of Annaba (Algeria)</td>
<td>Cu 4.56±0.69, Cr 15.88±0.59, Pb - -</td>
<td>Boumaza, 2014</td>
</tr>
<tr>
<td></td>
<td>Iskenderun Gulf (Turkey)</td>
<td>Cu 2.31±0.09, Cr - 0.44±0.03, Pb 0.30±0.03</td>
<td>Yuzereroglu et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Gulf of Suez (Egypt)</td>
<td>Cu 6.34±1.96, Cr 4.68±1.43, Pb 1.38±1.12, Pb 6.2±1.75</td>
<td>Hamed and Emara 2006</td>
</tr>
<tr>
<td></td>
<td>Gulf of Gaeta, Tyrrhenian Sea (Italy)</td>
<td>Cu 14.3±3.43, Cr 0.85±0.23, Pb 3.5±0.78, Pb 0.95±0.20</td>
<td>Conti and Cecchetti 2003</td>
</tr>
<tr>
<td><em>Patella vulgaris</em></td>
<td>Lebanese Coastal (Lebanon)</td>
<td>- - 2.16±0.26, Pb 1.55±0.34</td>
<td>Nakhle et al., 2006</td>
</tr>
<tr>
<td><em>Patella aspera</em></td>
<td>South coast (Portugal)</td>
<td>Cu 6.3±1.72, Cr - 3.5±0.57, Pb -</td>
<td>Cravo and Bebianno 2005</td>
</tr>
</tbody>
</table>
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.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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