



HEAVY METALS ASSESSMENT IN THE STRIPED VENUS CLAM, *CHAMELEA GALLINA*, IN EGYPTIAN FISHERIES AS POTENTIAL CANDIDATE FOR EXPLOITATION AND AQUACULTURE

Eman El-Wazzan^{1*}, Atif Salah², Mark Dimech³

¹ Invertebrates Lab, Aquaculture Division, National Institute of Oceanography and Fisheries, Qaiyet-Bay, El-Anfoushi, Alexandria, 21556, Egypt

Corresponding author email: emanelwazzan@yahoo.com

² General Authority for Fish Resources Development, 4 Tayaran St., Nasr City, Cairo, Egypt
atif_gafrd@yahoo.com

³ Food and Agriculture Organization, EastMed project, 1 Androu St., Athens, Greece
mark.dimech@fao.org

ABSTRACT

The Striped Venus Clam, *Chamelea gallina*, is one of the most important commercial bivalve species of the Verenedae family in the Mediterranean. It was suggested for the first time as potential candidate for exploitation from its natural fisheries and for possible aquaculture in Egypt (FAO EastMed 2014). The present study was conducted to investigate the heavy metals concentrations in *Chamelea gallina* from natural fisheries to evaluate its safety for human consumption as potential bivalve resource for exploitation in Egypt. *Chamelea gallina* was sampled from 12 transects representative of about 60 km along the Mediterranean coast between Rasheed (Rosetta) and Burullus from 31.45874 N and 30.50054 E to 31.59596 N and 30.94383 E. Clams soft tissues were analyzed for the levels of eight heavy metals; four hazardous metals [lead (Pb), cadmium (Cd), Mercury (Hg) and chromium (Cr)] and four essential metals [copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn)] using SHIMADZU Atomic Absorption Spectrophotometer. Results were compared to authorities' maximum permissible limits as set by different organizations of different countries on both dry weight (d.w) and wet weight (w.w.) basis. Results showed that the examined bivalve fisheries are considered clean compared to other areas of the world. They are considered safe for human consumption considering the FAO/WHO, USFDA and other regulations from different countries of the world. However, in few cases they failed to be approved for the European regulations which are the most strict legislations. Spatial distribution of heavy metals in clams across the investigated area showed that the clams are in general within safe limits especially in the western and middle parts of the investigated area from Rasheed eastwards. This zone have the maximum biomass of *C. gallina* as confirmed by a simultaneous fishery study (FAO EastMed 2014). This indicates that, *Chamelea gallina* in this zone is safe for human use and might provide promising fisheries for exploitation when toxic risk of the most hazardous heavy metals such Hg, Cu, Cr and Zn are concern. However, the eastern part of the studied area near Burullus must be monitored for Pb contamination and sources. Then, collection of *C. gallina* should be avoided when necessary.

Indexing terms/Keywords

Shellfish, Bivalve, Clam, Venus Clam, *Chamelea gallina*, Pollution, Biosafety, Heavy Metals, fishery, Aquaculture, Egypt

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1. INTRODUCTION

The edible bivalve molluscs, such as mussels (e.g. *Mytilus galloprovincialis*), oysters (e.g. *Ostrea* spp.), clams (e.g. *Donax* spp., *Tapes* spp. *Chamelea gallina*), cockles (e.g. *Acanthocardia* spp.) sustain important fisheries throughout the world, including the Mediterranean [1], where clams are the group with the highest landings.

The infaunal Striped Venus Clam, *Chamelea gallina*, is one of the most important commercial bivalve species of the Verenidae family in the Mediterranean [2], [3], [4]. It inhabits sandy bottoms of most of the Mediterranean inshore waters and is presently exploited in many countries such as Italy, Turkey, Greece, Spain and Morocco [5],[1] but has not been exploited in Egypt yet. *Chamelea gallina* was suggested for the first time as potential candidate for exploitation from its natural fisheries and for possible aquaculture in Egypt through a FAO-EastMed project [6]. Therefore, there was need to evaluate its suitability for human consumption for local use and for export. The possible heavy metal contamination of bivalve shellfish (e.g. oysters, clams, mussels and cockles) is a major food safety concern [7] due to the bivalves' high ability to bioaccumulate contaminants from the surrounding water over time [8], [9]. This might affect clam quality and suitability for human consumption.

Some commercial bivalve fisheries are polluted with different kinds of contaminants caused by the discharges of industrial and municipal effluents containing chemical and biological contaminants such as heavy metals, hydrocarbons organochlorines, pesticides, pathogenic bacteria and viruses [10], [11], [12], [13], [14], [15]. Recent studies in Egypt showed different levels of heavy metals contamination in edible bivalves [16], [17], [18], [19], [20] but no assessment have been made to *Chamelea gallina* due to the very recent interest in its exploitation. Therefore, there is need to assess the risk of any bivalve species (e.g. *Tapes decussatus*:[20]) that can be exploited in Egypt including the Striped Venus Clam *Chamelea gallina* to enhance public health protection.

Furthermore, assessment and classification of clam fisheries into approved or non approved harvesting areas are needed to dictate whether harvesting can be permitted from such fisheries and the areas should be periodically monitored. The present study is a part of a FAO EastMed project that investigates the potential exploitation of *Chamelea gallina* in Egypt [6]. Heavy metal concentrations were measured in *Chamelea gallina* from natural fisheries to evaluate its safety for human consumption as potential bivalve resource for exploitation in Egypt.

2. MATERIALS AND METHODS

2.1. Study Area

Sampling was conducted in an area between Rasheed and Burullus (Figure 1). The map shows the coastline between the two cities and the 5 and 10 m depth contour lines. The area consists of shallow water sandy and muddy bottoms of about 60 km in length along the Mediterranean coast between Rasheed and Burullus divided into 14 transects arranged at 4 km intervals along the coastline.



Figure 1. Map showing the area in which the study was conducted and the location of the start and end coordinates of the transects.

2.1.1. Sampling Sites

Chamelea gallina samples assigned for heavy metal analysis were selected from 12 transects representative of the studied area and extended from 31.45874 N and 30.50054 E to 31.59596 N and 30.94383 E (Table 1 shows individual samples information).

**Table 1.** Samples codes (Target Hauls Codes), geographical coordinates and depth of collection of the Striped Venus Clam *Chamelea gallina* for heavy metal analysis.

Target Hauls Code	PrHN°	GPS Coordinates				DEPTH (m)	
		START		END		START	END
		LatN	LongE	LatN	LongE		
A01.3	3	31.45874	30.50054	31.46004	30.5004	2.5	3
B01.1	4	31.46443	30.49735	31.46206	30.49884	6	5.2
A02.2	11	31.46659	30.54306	31.46746	30.54235	3	3
B02.2	14	31.47386	30.54023	31.47505	30.54325	5.2	5.6
A03.2	20	31.47787	30.59043	31.47795	30.59260	3.5	3.8
A03.3	21	31.48032	30.59477	31.48001	30.59580		3.9
A04.3	30	31.48686	30.63075	31.48693	30.62988	3.5	3.5
B04.3	33	31.49465	30.62794	31.49461	30.60320	5.4	5.3
C04.2	35	31.50511	30.62469	31.50566	30.62858	8.5	8
B05.1	40	31.51027	30.67956	31.50821	30.67992	6.3	
A06.1	46	31.50905	30.70628	31.51019	31.70818	3.2	3.3
A06.2	47	31.51151	30.71340	31.51224	30.71558	3.2	3.5
A06.3	48	31.51390	30.72055	31.51513	30.72255	3	3.4
B06.2	50	31.52013	30.76715	31.51854	30.70563	5.8	5.8
A08.1	64	31.53984	30.80530	31.53914	30.80721	3.5	3.2
B08.1	67	31.54062	30.79966	31.54445	30.80271	5.5	5.2
B08.2	68	31.54550	30.79247	31.54664	30.79865	6	5.5
B08.3	69	31.54501	30.78217	31.54621	30.78513	6.4	
B09.1	76	31.56298	30.84907	31.56477	30.85589	6.7	6
B09.2	77	31.55564	30.82896	31.55183	30.82973	6.4	5
B09.3	78	31.55484	30.82352	31.55260	30.82228	6.3	6
C10.3	90	31.57656	30.86324	31.57180	30.86166	8.8	8.5
B11.1	94	31.57673	30.89978	31.57768	30.90681	6.5	6.9
B12.1	103	31.58123	30.93228	31.58337	30.93468	5.9	6.2
B12.2	104	31.58313	30.93820	31.58193	30.93573	6.4	6.2
B12.3	105	31.58628	30.94037	31.58796	30.94400	6.5	6.7
C12.1	106	31.59367	30.94094	31.59596	30.94383	9	8.6

2.2. Heavy Metal Analysis

The striped Venus clam, *Chamelea gallina* soft tissues in each of 24 samples (50 clams or less/sample according to availability) were homogenized and analyzed for the levels of eight heavy metals; four hazardous metals [lead (Pb), cadmium (Cd), Mercury (Hg) and chromium (Cr)] and four essential metals [copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn)]. SHIMADZU Atomic Absorption Spectrophotometer AA-6800 equipped with GTA furnace and GVA cold vapor unit as well as flame unit was used for the analysis. Results were compared to authorities' maximum permissible limits as set by different organizations of different countries on both dry weight (d.w) and wet weight (w.w.) basis.

2.2.1. Clam Sample Processing

The clams assigned for heavy metal analysis were of different numbers according to the available number of clams from each station. Therefore, when clams from certain station were not enough, the available clams from the three replicates of

the same station were pooled and analyzed as one sample (e.g. A06.1+ A06.2+A06.3 and B09.2 + B09.3). Clams for each sample were cleaned, shucked with a plastic or Teflon knife to avoid contamination. Clam soft tissues pooled from each sample were homogenized and weighed (average weight 34.72 g; min 6.54 in sample B 12.1; max 105.62 g in sample A02.2). Similar aliquots of about 5 gm from each sample were dried at 105° C until constant weight for analyzing the seven metals other than Hg on dry weight basis (1 g powder used for each sample) and for calculation of water content and dry/wet ratio for further calculation of concentration in clams on wet weight basis. Additionally, 5 g aliquot of the wet sample was kept aside in plastic bottle for wet tissue Hg analysis (except in samples with total tissues weight available less than 10 g in which the sample was divided between drying and Hg) . Water content was calculated and heavy metals concentrations were calculated on both dry weight and wet weight basis for comparisons with permissible limits for different references and guidelines.

2.2.2. Heavy metal analysis in the NIOF Central Lab.

Samples were stored in polyethylene bags at -20° C until analysis. The extraction of Cu, Zn, Cr, Cd, Pb, Mn, and Fe as well as Hg was performed by using acid digestion bombs (with a Teflon cup) and concentrated nitric acid [21]. The following heavy metals in parts per million (Cu, Cr, Mn, Zn, Pb, Cd, Fe and Hg) were measured using SHIMADZU Atomic Absorption Spectrophotometer AA-6800 equipped with GTA furnace and GVA cold vapor unit as well as flame unit (detection limits in Table 2). Glassware utilized were soaked on aqua- regia, rinsed with milli-Q water .The reagent utilized were of high purity, appropriate for heavy metals analysis. The standards were prepared from stock solutions (Merk) A calibration curve of each heavy metals was prepared prior to every batch of analysis. The accuracy of measurements was tested using certified reference material ERM-CE278 (mussel tissue). Results (Table 2) were comparable to certified values and the standard deviations were low, showing feasibility of the used method. Detection limits are also included in Table 2. Co and Ni were not measured for technical reasons related to Central Lab at the time of analysis.

Table 2. Concentrations (average \pm standard deviation) of heavy metals mg/kg in the reference material ERM-CE278 (mussels tissue) as compared to certified values and the method detection limits.

Metal	ERM-CE278 (Mussel Tissue)		Detection limit (ppm)
	measured(mg/kg)	certified value(mg/kg)	
Cu	9.6 \pm 0.35	9.45 \pm 0.13	0.008
Cr	0.69 \pm 0.14	0.78 \pm 0.13	0.015
Mn	7.41 \pm 0.43	7.69 \pm 0.23	0.01
Zn	81.5 \pm 0.71	83.1 \pm 1.7	0.002
Pb	1.98 \pm 0.14	2.00 \pm 0.04	0.05
Cd	0.345 \pm 0.003	0.348 \pm 0.007	0.005
Fe			0.025
Hg	0.189 \pm 0.03	0.196 \pm 0.009	0.01 (ppb)

3. RESULTS

The main objective of the present study (this part of the FAO-EastMed project) is to investigate the heavy metal concentrations in the Striped Venus Clam, *Chamelea gallina* to evaluate its safety for human consumption as potential bivalve resource for exploitation from Egyptian fisheries.

Concentrations of eight heavy metals; four hazardous metals [lead (Pb), cadmium (Cd), Mercury (Hg) and chromium (Cr)]; and four essential metals [copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn)] were determined in tissues of the Striped Venus Clam, *Chamelea gallina* collected along the Mediterranean coast of Egypt in 12 transects from Rosetta to Burullus.

The measured concentrations/sample, averages and ranges (min-max) of the examined metals are represented as mg/kg dry weight and mg/kg wet weight (Table 3 and Table 4, respectively) and compared to lowest and highest permissible limits prescribed by legislations set by authorities from different countries for mollusks or bivalves.

3.1. Safety of *Chamelea gallina* For Human Consumption

The guidelines set by different organizations for permissible limits for sea food safety of the metals measured in the present study are recorded in Tables 5 and 6 on dry weight and wet weight bases, respectively.

Heavy metals concentrations measured in the present study (Tables 3 and 4) were compared to lowest (most strict) and the highest maximum permissible limits prescribed by different world authorities (Tables 5 and 6). Sites with heavy metal



concentrations that exceeded the lowest P.L. were marked by “**black bold face font**”. The concentrations that exceeded the highest permissible limits were marked with “**red bold face font**” in Tables 3 and 4, and red columns in Figures 2-5.

3.1.1 Comparison With Dry Weight Based Permissible Limits

When comparing the data of the present study (Table 3) to permissible limits on dry weight basis (see Table 5 for comparison), it was observed that:

Lead (Pb) concentration (av. 7.99 mg/kg d.w.) exceeded the lowest maximum permissible limits set for mollusks of 1.5 mg/kg d.w. prescribed by European communities [22] in all sites except 2 sites (B 02.2 and A 04.3) and the highest of 10 prescribed by Hong Kong Environmental Protection Department [23] (Table 5) in 5 sites out of 26 sites most of which located towards the east of the studied area reflecting the heavy contamination with Pb concentrated towards Burullus (**values in red bold face font** in Table 3 and red columns in Figure 2).

Cadmium concentrations exceeded the permissible limit set by USFDA 2007 [24] guidelines of 4 mg/kg dry weight (Table 5) in only two sites (B05.1 and B06.2). Values were 4.32 and 5.52 mg/kg d.w., respectively (Table 3; Figure 2). Cd exceeded the highest permissible limit of 5.5 mg/kg dry weight set by Marine Organism Pollution Assessment Standard (MOPAS) used in Guangdong Province, China [25] in only one site (B06.2). However, it exceeded the permissible limits of the European Community [22] of 1 g/kg d.w. (Table 5) in 20 out of 24 sites (**black bold face font** in Table 3) which reflects that the studied sites might be approved by American and other countries guidelines but not the European ones.

Chromium showed the same trend of cadmium with only one site exceeded the permissible limits of USFDA 2007 [24] of 13 mg/kg (37.30 mg/kg dry weight; Table 3; Figure 2) but most sites exceeded the 1 mg/g value set by FAO 1983 [26].

Mercury as the most potent toxic metal was under all permissible limits of 0.3 to 1 mg/kg dry weight in all sites showing that the studied area is completely clean of Hg contamination.

Similar observations were recorded for copper (Cu) and zinc (Zn) (Table 3) which were less than the lowest prescribed permissible limits of 30 and 150 mg/kg d.w., respectively (Table 5). This reflects that *C. gallina* fisheries are completely clean of the three metals.

There were no permissible limits for Mn and Fe to compare against so they will be compared to other studies regarding their recorded levels in *Chamelea gallina* as example of bivalves in Egypt rather than their risk to human health. The only record for permissible limits of Mn found in literature was 0.5 for WHO (1985) which was used by [27]. However, this limit is prescribed to drinking water. This guideline may not be convenient to use it for mollusks since the weakly intake of drinking water is different than the possible weekly consumption of bivalves which makes the risk to human health different if tolerable weekly intake was to be measured in both cases. For example, FAO/WHO (2004) [28] prescribed tolerable weekly intake for Pb as 0.3 mg/kg body weight but the maximum permissible concentration in fish was 0.4 mg/kg wet weight.

3.1.2. Comparison With Wet Weight Based Permissible Limits

When comparing the data of the present study (Table 4) to permissible limits on wet weight basis (Table 6), after results were corrected to clam water contents, it was observed that:

Pb concentration exceeded the lowest value for “maximum permissible limits” set for mollusks of 0.5 (mg/kg w.w) prescribed by FAO 1983 [25] (Table 5) in 17 out of 26 sites (**values in black bold face font** in Table 4). Only four sites all located on the eastern part of the studied area reflecting the heavy contamination with Pb concentrated towards Burullus (**values in red bold face font** in Table 4 and red columns in Figure 4).

Cd concentrations did not exceed the highest permissible limit of 2 mg/kg w.w. set by FAO/WHO (2011) [29]; CEP, (2002) [30] and Australian New Zealand food standard code [31] guidelines (Table 6; Figure 4). This reflects that all sites are approved clean of Cd according to the rules of these three organizations. However, Cd values exceeded the lowest (most strict) permissible limit of FAO 1983 [26] of 0.5 in 8 sites (**black bold face font** in Table 4). But since the most recent update of FAO/WHO 2011 [28] approved the 2 mg/kg limits. It should be used instead of FAO 1983 [26] which means all sites rendered clean of Cd based on the above mentioned three organizations from Europe and Australia.

Cr concentration on wet weight basis showed the same trend the dry weight values with only one site (B02.2) with Cr concentration (9.04 mg/kg w.w.) that exceeded the permissible limits of FAO 1983 [26] that prescribed 1 mg/g w.w. (Table 4; Figure 4).



Table 3. Heavy metals concentrations on dry weight basis in the soft tissue of the Striped Venus Clam, *Chamelea gallina* collected from different stations along the Mediterranean coasts from Rasheed to Burullus.

Sample Code	Heavy Metals Concentration (Dry Samples mg/kg)							
	Pb	Cd	Hg	Cr	Cu	Zn	Mn	Fe
A 01.3	2.55	1.44	0.04	2.50	12.19	55.34	56.88	1134.46
B 01.1	3.95	2.01	0.05	2.85	15.38	80.79	28.82	468.35
A 02.2	3.40	1.17	0.04	3.50	7.38	56.72	16.97	423.39
B 02.2	1.30	3.08	0.07	37.30	18.78	79.85	35.03	1424.19
A 03.2	5.06	1.20	0.06	3.26	13.47	55.45	25.63	966.48
A 03.3	6.28	0.60	0.03	1.48	10.06	45.18	18.35	379.35
A 04.3	0.59	0.99	0.08	1.66	6.63	36.32	15.54	655.13
B 04.3	4.32	1.56	0.05	1.57	5.80	35.59	11.43	383.13
C 04.2	5.49	2.70	0.02	2.95	13.06	66.79	27.92	1160.85
B 05.1	3.47	4.32	0.03	3.17	13.09	76.27	41.68	1213.91
A 06.1,2,3	6.25	3.99	N.D	2.56	8.53	57.68	24.86	863.61
B 06.2	3.03	5.52	0.09	1.57	9.90	64.42	25.82	774.07
A 08.3	6.02	2.20	0.03	6.07	12.46	86.84	29.70	1593.10
B 08.1	9.00	3.27	0.12	3.92	13.43	84.35	28.96	1240.88
B 08.02	10.76	3.22	0.06	4.77	16.90	79.35	49.41	2077.11
B 08.3	4.34	3.19	0.03	4.01	12.18	69.67	31.34	1075.36
B 09.1	5.81	0.76	0.10	1.94	7.85	41.43	18.60	606.30
B 09.2,3	7.06	3.45	0.04	2.91	12.62	82.27	17.22	875.33
C 10.3	13.55	1.75	0.07	2.93	12.15	69.22	13.41	366.73
B 11.1	19.77	2.78	0.06	4.74	17.37	107.17	30.20	1498.94
B 12.1	35.60	2.88	0.10	5.18	9.39	71.24	30.70	1430.57
B 12.2	9.94	2.68	0.08	4.14	20.39	69.46	32.36	1808.88
B 12.3	9.95	1.52	0.01	4.23	12.15	72.78	21.67	1031.11
C12.1	14.35	0.54	0.13	3.07	2.85	30.25	6.25	205.91
Average	7.99	2.37	0.06	4.68	11.83	65.60	26.62	986.00
Min	0.59	0.54	0.01	1.48	2.85	30.25	6.25	205.91
Max	35.60	5.52	0.13	37.30	20.39	107.17	56.88	2077.00

*Min to max
P.L. set for
mollusks

1.5 to 10 1 to 5.5 0.3 to 1 1 to 13 30 - 70 1000 NP NP

* Max permissible limits (P.L.) range (min – max) set by organizations from different world countries and authorities; NP means no permissible levels found for these metals.



Table 4. Heavy metals concentrations on wet weight basis in the soft tissue of the Striped Venus Clam, *Chamelea gallina* collected from different stations along the Mediterranean coasts from Rasheed to Burullus.

Site Code	Heavy Metals Concentration (mg/kg wet weight)							
	Pb	Cd	Hg	Cr	Cu	Zn	Mn	Fe
A 01.3	0.75	0.42	0.010	0.73	3.57	16.20	16.65	332.06
B 01.1	0.86	0.44	0.012	0.62	3.35	17.63	6.29	102.19
A 02.2	0.63	0.22	0.007	0.65	1.38	10.57	3.16	78.92
B 02.2	0.32	0.75	0.016	9.04	4.55	19.35	8.49	345.08
A 03.2	1.08	0.26	0.013	0.70	2.87	11.82	5.46	206.05
A 03.3	1.58	0.15	0.009	0.37	2.53	11.37	4.62	95.44
A 4.3	0.10	0.17	0.014	0.28	1.13	6.19	2.65	111.57
B 04.3	0.98	0.35	0.011	0.36	1.31	8.07	2.59	86.82
C 04.2	1.12	0.55	0.004	0.60	2.68	13.69	5.72	237.86
B 05.1	0.60	0.75	0.005	0.55	2.26	13.16	7.19	209.52
A 6.1,2,3	0.92	0.59	N.D	0.38	1.26	8.51	3.67	127.38
B 06.2	0.44	0.79	0.013	0.23	1.42	9.26	3.71	111.31
A 08.3	0.94	0.34	0.005	0.95	1.95	13.59	4.65	249.32
B 08.1	1.34	0.49	0.018	0.58	1.99	12.53	4.30	184.27
B 08.02	1.53	0.46	0.008	0.68	2.40	11.26	7.01	294.74
B 08.3	0.88	0.65	0.005	0.81	2.47	14.12	6.35	217.98
B 09.1	0.76	0.10	0.013	0.25	1.03	5.43	2.44	79.49
B 09.2,3	1.23	0.60	0.008	0.51	2.20	14.36	3.00	152.75
C 10.3	1.77	0.23	0.009	0.38	1.59	9.05	1.75	47.93
B 11.1	2.55	0.36	0.008	0.61	2.24	13.85	3.90	193.66
B 12.1	6.86	0.55	0.018	1.00	1.81	13.72	5.91	275.53
B 12.2	2.32	0.63	0.018	0.97	4.76	16.23	7.56	422.74
B 12.3	1.94	0.30	0.003	0.83	2.37	14.22	4.24	201.48
C12.1	2.77	0.10	0.025	0.59	0.55	5.84	1.21	39.78
Average	1.43	0.43	0.011	0.94	2.24	12.08	5.11	183.49
Min	0.10	0.10	0.003	0.23	0.55	5.43	1.21	39.78
Max	6.86	0.79	0.025	9.04	4.76	19.35	16.65	422.74

* Min to max P.L. set for mollusks

0.5- 2 0.5-2 0.5 1 3.0-30 30-100 NP NP

* Max permissible limits (P.L.) range (min – max) set by organizations from different world countries and authorities; NP means no permissible levels found for these metals.



Hg (average concentration= 0.01 mg/kg w.w.), and Zn (average concentration= 12.08 mg/kg w.w.) were less than the lowest prescribed permissible limits (Table 6) of 0.5 and 30 mg/kg w.w., respectively (Table 4; Figures 4 and 5). This reflects that *C. gallina* fisheries are completely clean of the two metals.

Cu (2.24 mg/kg w.w.) was lower than the higher permissible limit set for wet weight of 30 mg/kg w.w. by CEP (2002) [30], but few sites exceeded the FAO/WHO (2011) guidelines [29] of 3.0 mg/kg w.w. (**bold face font in Table 4**). The examined area is considered clean considering Cu contamination.

When values were above permissible limits for European Union, concentrations of all heavy metals in *Chamelea gallina* were below maximum permissible limit set by other countries (**values in Red Font bold face font** in Table 3 and Table 4). Only Pb in few stations was higher than these values when values were corrected for water contents and represented as wet weight.

3.2. Spatial Distribution of Heavy Metals in the Studied Area

Looking at the possible relation of the heavy metals concentration and collection site (Figures 2-5), it was observed that there was no significant relation between any of the measured heavy metals and collection site. However, some metals showed high concentration in certain sites with no similar increase in other metals.

For example, Cu showed highest value at the two ends of the collection range but the middle sites had moderate values that were not significantly different from highest values. Mn highest value was in the west and the rest were moderate (Figures 3 and 5).

Cr showed only one high site (B 02.2) that exceeded the permissible limits which would be considered as outlier from statistical point of view (Figures 2 and 4). The only metal that showed significant difference among sites was Pb which showed higher values eastwards (Figures 2 and 4).

In general, there is no certain trend of heavy metal concentration in the studied area and it is considered clean except for the eastern part which has higher Pb concentrations.

3.3. Comparison of the Present Study With Other Studies

Tables 7 and 8 show comparisons of heavy metals concentrations data of the present study with others from literature on both dry weight (d.w.) and wet weight (w.w.), respectively.

For *Chamelea gallina*, it was observed that Pb, Cd, and Cr concentrations (mg/kg d.w) measured in the present study were higher than those measured in *C. gallina* from Spain [32]. However, Hg, Cu and Zn concentrations in that study were higher than those observed in the present study (Table 7). Similarly, lower Pd, Cd, Cr and Fe but higher Cu concentrations were measured as mg/kg w.w. in *C. gallina* collected from Turkey [33] than those recorded in the present study (Table 8).

For other bivalves based on dry weight comparisons (Table 7), nine stations were studied from Rosetta (Rasheed in the present study) to Port Said cities, Egypt using pooled samples of different bivalve species that represented biodiversity of each station [18]. The study did not include *C. gallina* among the recorded bivalves. However, all the heavy metals measured were lower than those observed in the present study (Table 7).

In another study heavy metals were examined in *Donax trunculus* along the Mediterranean coast of El-Gamil zone west of Port Said, Egypt (between lat. 31° 10' – 31° 20' N and long. 32° 00' - 32° 20') which is east of the area studied in the present study [19]. However, all metals showed lower values than those measured in the present study (Table 7). Data in Table 7 show lower Pb, Cd, Cu, Fe, Zn but higher Hg in two bivalve species, *Mactra* spp. and *Mytilus* sp. from Alexandria, Egypt [16].

Mytilus galloprovincialis from Apulian Mediterranean coast of Italy had much lower Pb, Cd and Cr levels [34] than those measured in the present study. However, Hg ranges were higher and Cu ranges were more or less similar to the ranges measured in the present study. Wild mussels, *Perna viridis*, from the west coast of Malaysia [35] had Cu, Fe and Zn levels within the ranges measured in the present study but closer to the upper limit and less Cd range (Table 7).

Comparing with data based on wet weight (mg/kg w.w.) from other studies (Table 8), it was observed that heavy metal concentrations in the carpet shell clam *Tapes decussatus* from six Egyptian fisheries [20] showed lower Pb concentration ranges than those in observed in *Chamelea gallina* of the present study (0.01 – 3.03 and 0.10 - 6.86 mg/kg w.w., respectively) and similar Cu ranges (0.46 - 4.94 and 0.55 - 4.76, respectively). In contrast, higher Cd concentrations were found in *Tapes decussatus* (0.01 - 2.24 mg/kg w.w.) than those found in the present study (0.10 - 0.79 mg/kg w.w.; Table 8). The concentrations of Pd, Cd and Hg in Cockles from Malaysia [36] were higher than observed in the present study (Table 8).



Table 5. Comparison of concentrations of heavy metal in *C. gallina* (average, min and max.) with the guidelines of permissible limits of seafood for human safety by different countries based on dry weight permissible limits.

N O	Legislation Organization and target organisms	Permissible Limits of Heavy Metals Concentration on dry weight basis (mg/kg d.w.)							Reference and organism studied; Country	
		Pb	Cd	Hg	Cr	Cu	Zn	Mn		Fe
1	FAO/WHO, 1984 [43], 2011 [29]: Fish & mollusks	0.3**- 6	2*, 1**	0.5*	50	10 to 100	150	5.4**		
2	*Europe (European Communities, 2001) [22]: mollusks	1.5	1	0.5					Usero <i>et al.</i> 2005 [32]: mollusk <i>Chamelea gallina</i> ; Spain	
3	* Abbott <i>et al.</i> , 2003 [31]: mollusks	2	2	0.5					Usero <i>et al.</i> 2005 [32]: mollusk <i>Chamelea gallina</i> ; Spain	
4	*HKEPD, 1997 [23]: marine mollusks	6	2	0.5	1				Zhan qiang <i>et al.</i> 2001 [54]: edible bivalve; China	
5	*MOPAS [25]: marine mollusks	10	5.5	0.3	5.5				Zhan qiang <i>et al.</i> 2001 [54]: edible bivalve; China	
6	*USFDA, 2007 [24]: molluscan shellfish	1.7	4	1	13				Yap <i>et al.</i> 2008 [35]: mussels, Malaysia	
7	* FAO 1983 [26]: mollusks	2-5.5	2	0.5	1	70	1000		Spada <i>et al.</i> 2013 [34]: mussel <i>Mytilus galloprovincialis</i> ; Italy	
	Max permitted range set for mollusks	1.5 to 10	1 to 5.5	0.3 to 1	1 to 13	30 to 70	150-1000	NP	NP	Different authorities and organizations
	Present study Average conc.	7.99	2.37	0.06	3.50	11.83	65.60	26.62	986	values in bold face font are above max permissible limits for mollusks
	Present study min conc.	0.59	0.54	0.01	1.48	2.85	30.25	6.25	206	
	Present study max conc.	35.60	5.52	0.13	9.04	20.39	107.17	56.88	2077	

* values were set for mollusks and bivalves; ** values were set for fish; NP means no permissible levels found for these metals



Table 6. Comparison of concentrations of heavy metal in *C. gallina* (average, min and max.) with the guidelines of permissible limits of seafood for human safety by different countries based on wet weight permissible limits.

Legislation Organization	Permissible Limits of Heavy Metals Concentration on Wet weight basis (mg/kg w.w.)								Reference # and organism studied; Country
	Pb	Cd	Hg	Cr	Cu	Zn	Mn	Fe	
*, ** FAO/WHO 2011 [29]; fish and bivalve	0.3**	2*	0.5**		3.0				
* EU 2006 (EC Reg.n. 1881/2006) [37]; mollusks	1.5	1	0.5						Spada <i>et al.</i> 2013 [34]; mussel, <i>Mytilus galloprovincialis</i> ; Italy
*, **FAO/WHO 2004 [28]; fish and shellfish	1.5*	0.05**	0.5*						Alina <i>et al.</i> , 2012 [36]: fish and shellfish
*Australia New Zealand Food Standards Code (Abbott <i>et al.</i> , 2003) [31]; mollusks	2	2	0.5						Usero <i>et al.</i> 2005 [32]: mollusk <i>Chamelea gallina</i>
*CEP, 2002 [30]; mollusks		2			30	40-100			Yap <i>et al.</i> 2008 [35]: mussels, Malaysia
*Europe (European Communities, 2001) [22]; mollusks	1.5	1	0.5						Usero <i>et al.</i> 2005 [32]: mollusk, <i>Chamelea gallina</i>
**HKEPD, 1997 [23]; fish	6	2	0.5	1					Nor Hasyimah <i>et al.</i> 2011 [53]: fish; Malaysia
** MOPAS [25]; fish	10	5.5	0.3	5.5					Nor Hasyimah <i>et al.</i> 2011 [53]: fish; Malaysia
**USFDA, 1990;1993 [39];[40]; Fish	1.7	3.7		12 to 13					Nor Hasyimah <i>et al.</i> 2011 [53]: Fish; Malaysia; Yap <i>et al.</i> 2008 [35]: mussels, Malaysia
Fourteenth Schedule (Regulation 38); Malaysian Food Regulation (1985)* [38]	2***	1***	0.5***		30	100			Alina <i>et al.</i> , 2012 [36]: fish and shellfish; Yap <i>et al.</i> 2008 [35]: mussels, Malaysia
*FAO 1983 [26]; mollusks	0.5	0.5	0.5	1	30	30			Spada <i>et al.</i> 2013 [34]; mussel <i>Mytilus galloprovincialis</i> ; Italy
Max permitted range set for mollusks	0.5 - 2	0.5 - 2	0.5	1	3.0 to 30	30 to 100	NP	NP	Different authorities and organizations
Present study Average conc.	1.43	0.43	0.01	0.94	2.24	12.08	5.11	183	values in bold face font are above max permissible limits for mollusks
Present study min conc.	0.10	0.10	0.00	0.23	0.55	5.43	1.21	40	
Present study max conc.	6.86	0.79	0.02	9.04	4.76	19.35	16.65	423	

* values were set for mollusks and bivalves; ** values were set for fish; ***values are in mg/g; NP means no permissible levels found for these metals.

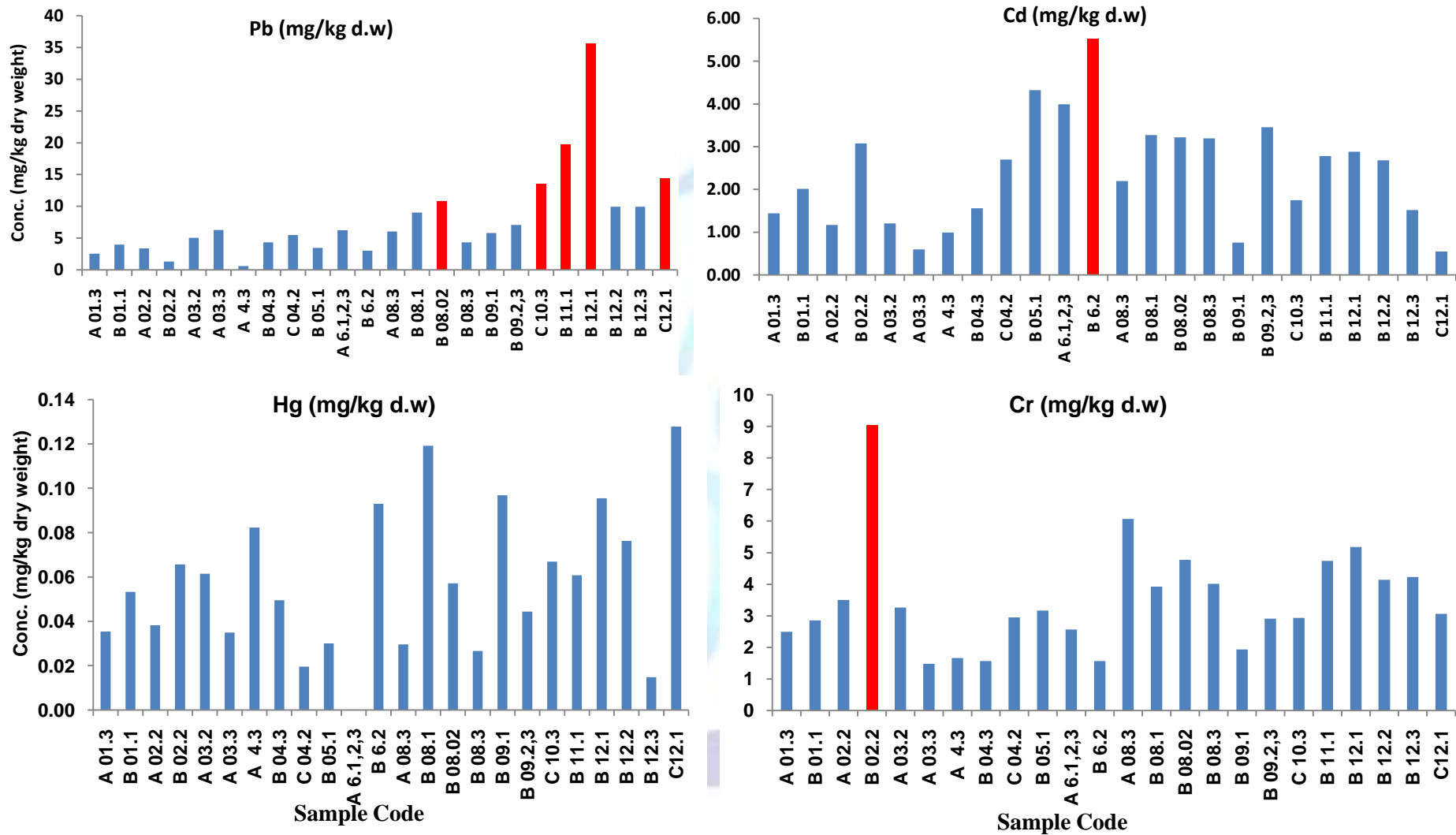


Figure 2. Concentrations (mg/kg d.w.) of Pb, Cd, Hg and Cr in the striped Venus clam, *Chamelea gallina*, collected from 12 transects along Egyptian Mediterranean coast from Rosetta to Burullus. Data points in red color exceed the maximum permissible limits for the concerned metal set by all mollusk sanitation guidelines.

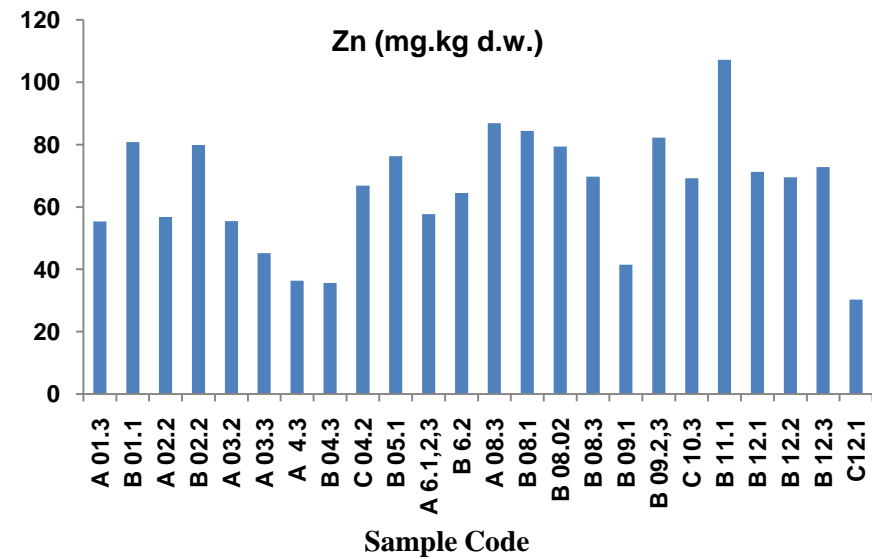
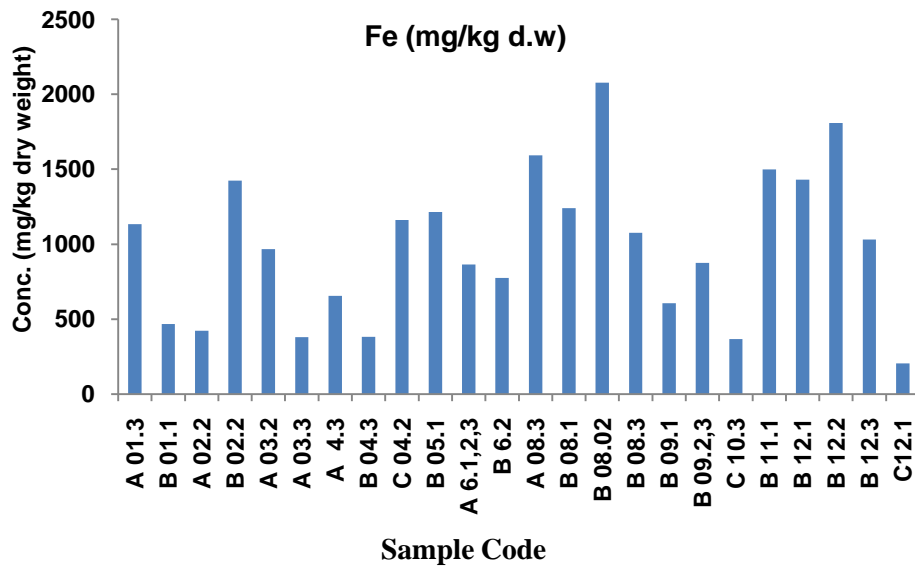
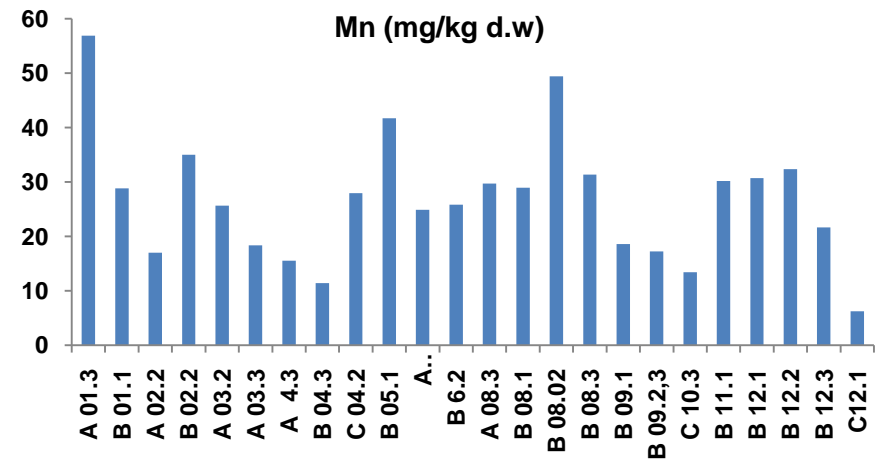
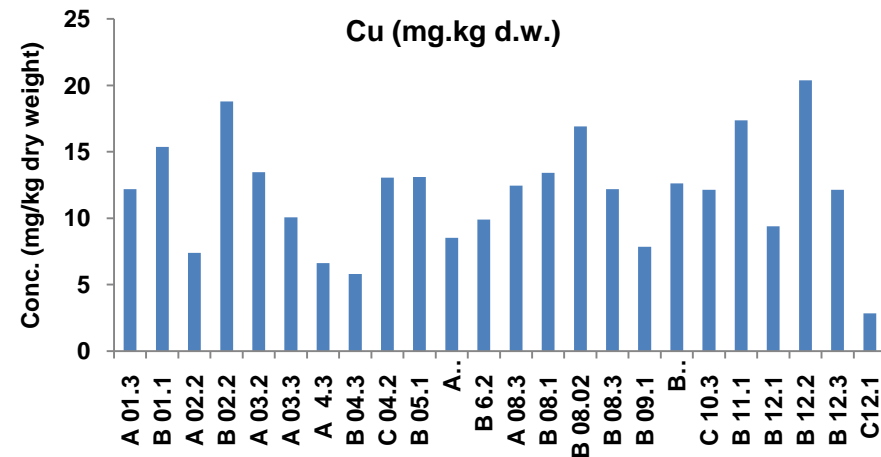


Figure 3. Concentrations (mg/kg d.w.) of Cu, Mn, Fe and Zn in the striped Venus clam, *Chamelea gallina*, collected from 12 transects along Egyptian Mediterranean coast from Rosetta to Burullus. Data points in red color exceeded the maximum permissible limits for the concerned metal set by all mollusk sanitation guidelines.

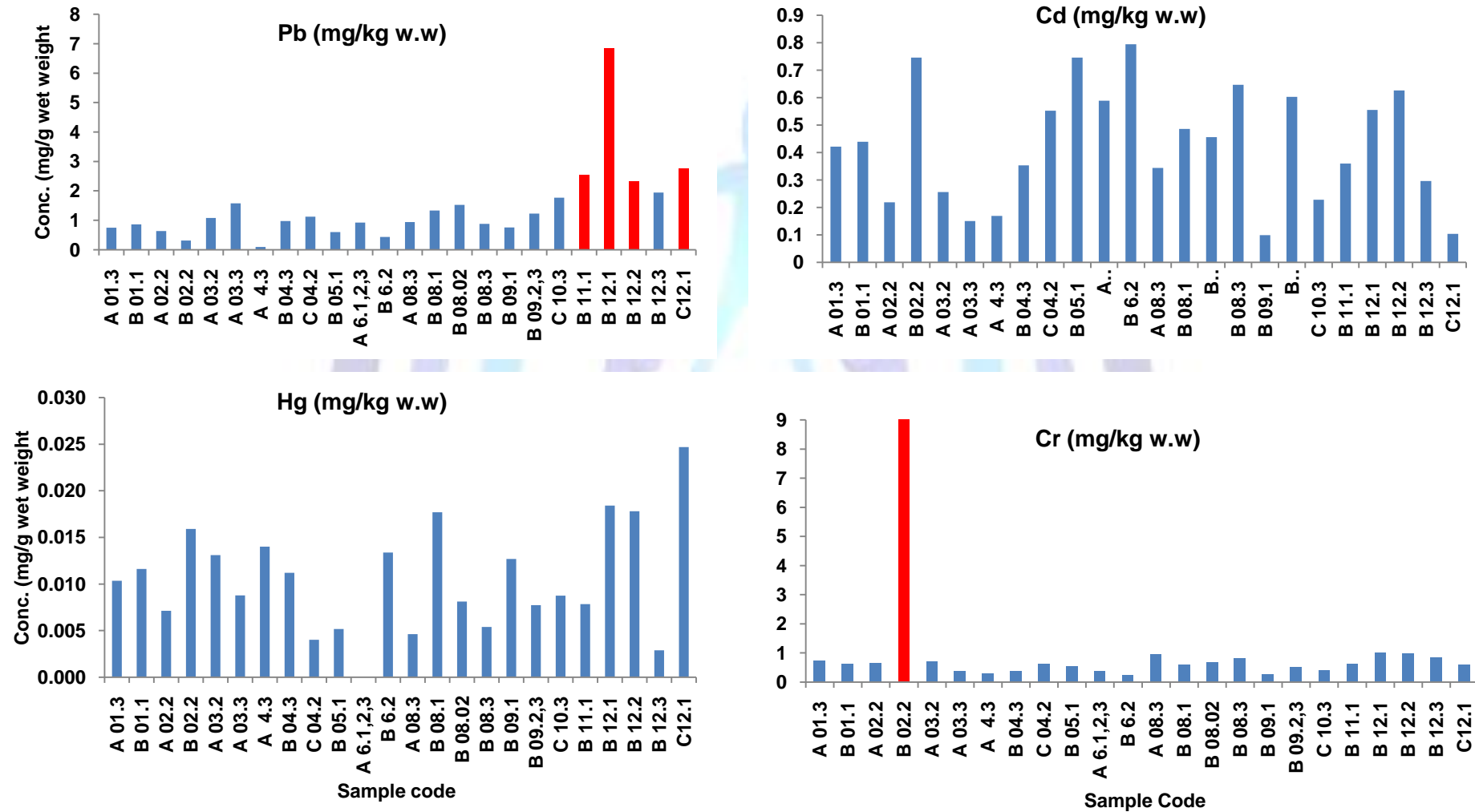


Figure 4. Concentrations (mg/kg w.w.) of Pb, Cd, Hg and Cr in the striped Venus clam, *Chamelea gallina*, collected from 12 transects along Egyptian Mediterranean coast from Rosetta to Burullus. Data points in red color exceed the maximum permissible limits for the concerned metal set by all mollusk sanitation guidelines.

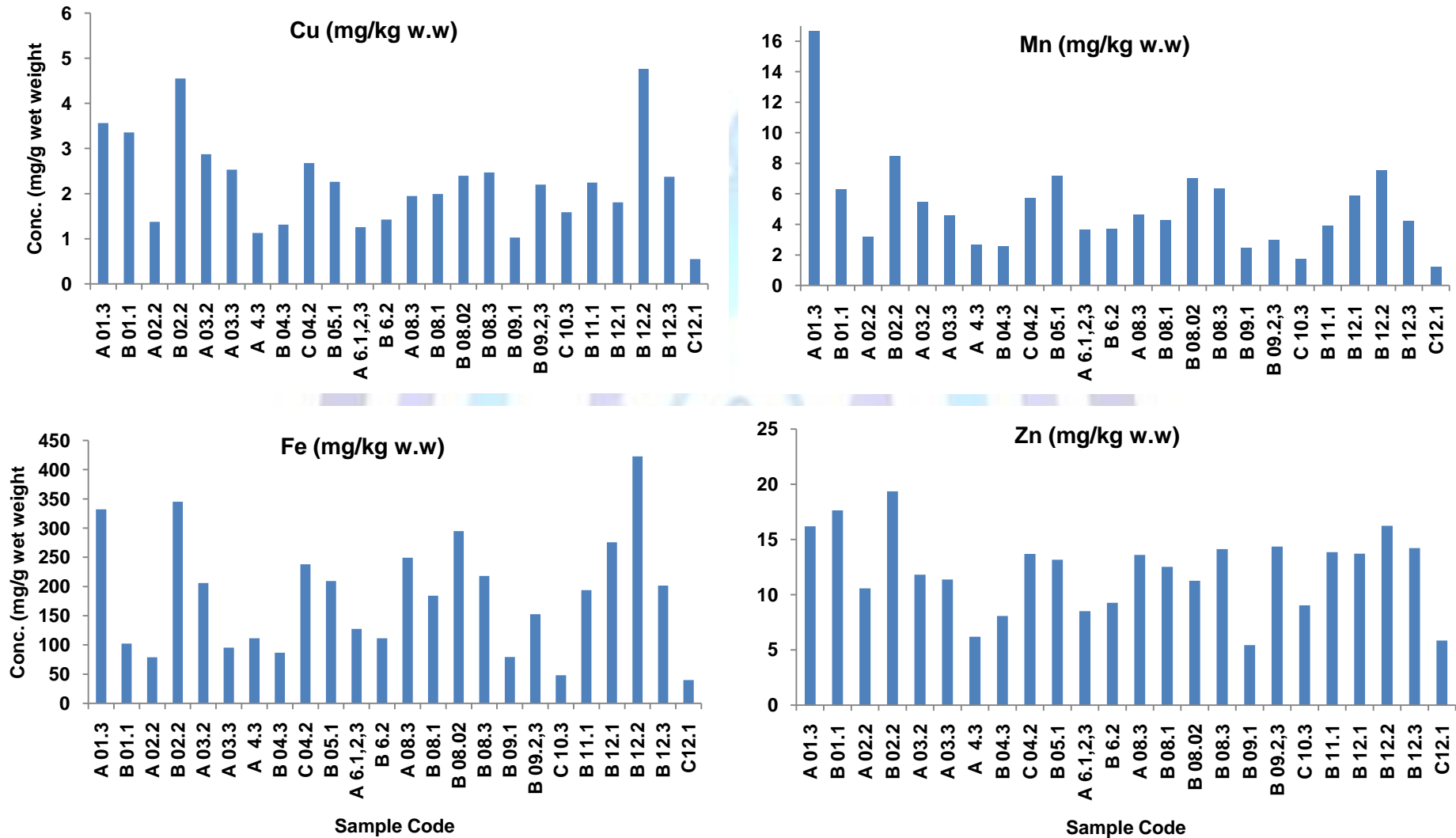


Figure 5. Concentrations (mg/kg w.w.) of Cu, Mn, Fe and Zn in the striped Venus clam, *Chamelea gallina*, collected from 12 transects along Egyptian Mediterranean coast from Rosetta to Burullus. Data points in red color exceed the maximum permissible limits for the concerned metal set by all mollusk sanitation guidelines.



Table 7. Comparison of concentrations of heavy metal in *C. gallina* (average, min and max.) in the present study on dry weight basis with other bivalve studies from different countries.

Reference	Country (area)	Organism	Heavy Metals Concentrations Ranges, means (mg/kg d.w.) in mollusks from different countries							
			Pb	Cd	Hg	Cr	Cu	Mn	Fe	Zn
Present study (FAO – EastMed Project)	Egypt (Rasheed to Burullus)	Striped Venus Clam, <i>Chamelea gallina</i>	7.99 (0.59 - 35.60)	2.37 (0.54 - 5.52)	0.06 (0.01 - 0.13)	4.68 (1.48 - 37.30)	11.84 (2.85-20.39)	26.62 (6.25 - 56.88)	986 (206 - 2077)	65.6 (30.25 - 107.17)
Spada <i>et al.</i> , 2013 [34]	Italy	<i>Mytilus galloprovincialis</i>	(0.37 - 3. 25)	(0.38 - 1.84)	(0.1 - 0.81)	(0.96-9.46)	(4.66-19.22)			
El-Serehy <i>et al.</i> , 2012 [19]	Egypt (west Port Said)	<i>Donax trunculus</i>	(5.6 - 9.2)	(1.6 - 2.4)			(3.2 - 4.8)	(4.8 - 8.4)	(47.2 - 66.4)	(22 - 36.4)
Ahdy <i>et al.</i> , 2007 [16]	Egypt (Alexandria)	<i>Macra spp.</i>	0.085 (0.03 - 0.17)	0.06 (0.03 - 0.29)	0.15 (0.011 - 0.33)	0.85 (0.7 - 17)	1.0 (0.5 - 2.1)		10.5 (7.7 - 18.7)	22 (18 - 35)
Ahdy <i>et al.</i> , 2007 [16]		<i>Mytilus spp.</i>	0.07 (0.06-1.08)	0.048 (0.031 - 0.18)	0.12 (0.061-0.270)	0.79 (0.6 - 1.1)	1.3 (0.7 - 25)		9.4 (7.2-16.1)	18 (17-20)
El Nemr <i>et al.</i> , 2012 [18]	Egypt (Alexandria to port Said)	Pool of different bivalves available	0.25 ± 0.15	0.09 ± 0.04		8.49 ± 5.19	3.82 ± 2.21			21.87 ± 21.38
Usero <i>et al.</i> , 2005 [32]	Spain	Striped Venus Clam, <i>Chamelea gallina</i>	1.3	0.33	6.4	0.7	38			72
Usero <i>et al.</i> , 2005 [32]	Spain	<i>Donax trunculus</i>	3.6	0.19	0.12	1.2	175			107
Yap <i>et al.</i> , 2008 [35]	Malaysia	Wild mussels, <i>Perna viridis</i>		(0.51 - 2.90)			(12.61 - 15.01)		(385 – 1271)	(81.74 - 106.6)



Table 8. Comparison of concentrations of heavy metal in *C. gallina* (average, min and max.) in the present study on wet weight basis with other bivalve studies from different countries.

Reference	Country (area)	Organism	Heavy Metals Concentration Range, mean (mg/kg w.w.) in different mollusks and permissible limits used						
			Pb	Cd	Hg	Cr	Cu	Mn	Fe
Present study (FAO – EastMed Project)	Egypt	Striped Venus Clam, <i>Chamelea gallina</i>	1.43 (0.10 - 6.86)	0.43 (0.10 - 0.79)	0.011 (0.003 - 0.025)	4.68 (1.48 - 37.3)	2.24 (0.55 - 4.76)	5.11 (1.21 - 16.65)	184 (39.78 - 423)
El-Wazzan <i>et al.</i> , 2013 [20]	Egypt	Carpet shell clam, <i>Tapes decussatus</i>	(0.01 – 3.03)	(0.01 - 2.24)			(0.46 - 4.94)		
Çolakoğlu <i>et al.</i> , 2010 [33]	Turkey	Striped Venus Clam, <i>Chamelea gallina</i>	(0.18 - 3.24)	(0.04 - 0.69)		(0.08 - 1.25)	(0.71 - 5.30)		(2.46 - 89.73)
Alina <i>et al.</i> , 2012 [36]	Malaysia	Cockles	0.19 - 0.28	25.1 - 47	3.6 - 6				



4. DISCUSSION

Based on the results of FAO East Med 2014 in a parallel assessment of resources, *Chamelea gallina* could be promising for exploitation from Egyptian waters [6]. Most biomass is located on the Western side of the investigated area close to the fishing port of Rasheed, making the area more easily reachable from this fishing port [6]. In theory the export of clams could be possible. However, several countries require special regulations for the import of bivalve species. For the biggest export market, that is, the European Union, the countries within the EU require that for any importation of bivalves the marine waters have to be classified based on the water quality parameters. Since bivalves are filter feeders they tend to bio-accumulate all sorts of contaminants, from bacterial and viral to heavy metals. Therefore, most of the potential production would probably be more suited for local consumption in Egypt until complete evaluation of resources and bio safety is accomplished.

Mollusks are able to accumulate heavy metals and impose health hazard to consumers. Heavy metals are classified into toxic and non-toxic groups. From human health risk point of view, the most toxic metals are As, Pb, Cd, Hg and Cr. Bioaccumulation of these metals in tissues leads to cellular damage and dysfunction of many organs and may lead to death. The most important of these are Pb, Cd and Hg as they are potent toxins. Therefore, strict legislations for maximum permissible limits (P.L.) have been set for human consumption by different authorities and organizations (e.g. [26]; [38]; [39]; [40]; [41]; [24]; [22]; [42]; [37]; [43]; [44]; [28]; [29]). In contrast, Cu, Mn, Fe and Zn and Co are essential metals and play vital role in metabolic functions at low levels [45]. However, if they bio-accumulate to reach toxic levels, they pose health risk [46].

Several studies monitored heavy metals in bivalves in Egypt as bioindicators of pollution and for assessing their suitability for human consumption ([47]; [48]; [16]; [18]; [19]; [20]).

Heavy metals have increased in the Egyptian marine environment in the past few decades due to disposal of large amounts of variety of contaminants from agricultural drainage, domestic wastes, shipping activities, sewage and nutrients ([49]; [10]; [50]). The deterioration of water quality in Egyptian fisheries and their contamination with heavy metals raised the safety concern of using fish and shellfish from Egyptian fisheries for human consumption.

The danger of heavy metals arises from the fact that they are persistent in the marine environment and cannot be metabolized by the body. They accumulate at a rate faster than the body can eliminate them causing severe danger to health leading to DNA damage and cancer in some cases due to their high toxicity ([51]; [52]).

Results show that the examined fisheries are considered clean compared to other areas of the world as Malaysia [36]. *C. gallina* examined in the present study was clean especially for contamination with Hg and Cu compared to bivalve studies in Spain and Turkey ([32]; [33]). However, contamination with Pb, Cd, Cr, and Fe in *C. gallina* exceeded those observed in some bivalves in Spain, Turkey and Italy ([32]; [33]; [34], respectively).

Furthermore, heavy metals measured in the present study were higher than those measured in other Egyptian fisheries such as Alexandria [16], Alexandria to west Port Said [18] and west Port Said [19]. In contrast, only Pb was higher in the present study than in another study in which *Tapes decussatus* collected from Alexandria, Ismailia and Damietta was examined [20]. In that study, Cd values were much higher than those observed in the present study and exceeded permissible limits set by FAO (1983) of 0.5 mg/kg w.w. [16] only in Alexandria (El-Max) and Ismailia (Timsah Lake) but not Damietta. However, it exceeded the 2 mg/kg w.w. prescribed by other regulations (e.g. [29]; [30]; [31] for mollusks in only samples of El-Max site of Alexandria.

In general, although the concentrations of some heavy metals measured in the present study were higher than those of other studies, the differences were not exaggerated and the measured levels were still within the permissible limits of some countries. Even when values were above permissible limits for European Union, concentrations of all heavy metals in *Chamelea gallina* were below maximum permissible limit set by other countries. Only Pb was higher than these values (5 sites and 4 sites for the dry weight values and when values were corrected for water contents and represented as wet weight, respectively). Therefore, Rasheed which has the maximum biomass of *C. gallina* [6], is completely clean and Burullus is considered heavily contaminated with Pb.

In agreement with the present study, studying levels of Cd, Cu, Ni, Pb and Zn in several bivalves collected along the Egyptian Mediterranean coast from Alexandria to Port Said showed that these metals do not cause adverse effect for either low or high shellfish consumers [18]. Cr was the only metal that had risk of causing health problems for heavy shellfish consumers. These results show that open water fisheries in the present study and other studied are less contaminated with heavy metals than closed ones as lake Timsah and El-Max area of Alexandria [20].

All these results from Egyptian fisheries reflect that most Egyptian bivalve fisheries are considered relatively clean considering the FAO/WHO, USFDA and other regulations from different countries of the world ([29]; [30]; [31]). However, in few cases they fail to be approved for the European regulations ([22]; [37]) which are the most strict legislations (e.g. some sites in the present study for Pb in *C. gallina* and for Pb and Cd in *Tapes decussatus* [20].

This indicates that, *Chamelea gallina* collected from the studied areas should be safe for human use and might provide promising fisheries when toxic risk of the most hazardous heavy metals such Hg, Cu, Cr and Zn are concern. However, the eastern part of the studied area must be monitored for Pb contamination and sources. Then, collection of *C. gallina* should be avoided when necessary. Taking in consideration the fact that bivalve from these fisheries are safe for human use, if the product is successfully introduced on the local markets, there will be a need to develop biomonitoring program for the bivalve health and suitability to human consumption as part of the fisheries management plan.



5. ACKNOWLEDGMENT

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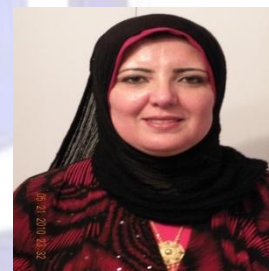
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Author' biography with Photo

Dr. Eman El-Wazzan is experienced marine biologist and marine environment scientist working as Research Doctor (Ph.D. Lecturer Researcher) at the Marine Invertebrates Lab, Aquaculture Division of the National Institute of Oceanography and Fisheries (NIOF). She has eleven years of experience in marine environmental sciences especially marine pollution and marine biotechnology and twelve years of experience in invertebrate aquaculture especially bivalve aquaculture. She has earned her B.Sc. degree in general oceanography and M.Sc. in Chemical Oceanography with honor from Alexandria University, Egypt. She completed her Ph.D. studies in bivalve culture and molecular biology in Florida Institute of Technology, Melbourne, USA and earned her Ph.D. degree in Biological Sciences (Marine Biology) in 2008.



Post-doctoral studies involved evaluation of bivalves in Egyptian fisheries and stress-related biomarkers. She introduced bivalve aquaculture in Egypt in 2009 and initiated and managed bivalve aquaculture research program at NIOF when she secured fund and acted as a principal investigator of a joint Egypt-US project funded by STDF, Egypt and USDA, USA and other national projects. She participated as PI, team leader or team member in 8 international and national projects all dealt with invertebrate ecology, biodiversity, biotechnology and aquaculture (bivalves, sponges and sea cucumber). She was contracted as National Consultant by FAO organization in a FAO- EastMed-GAFRD project, 2013-2014. She is an editor in *World Life Sciences (WLS)-Global Technology Press*. She completed project management professionals courses (PMP) and preparing to be PMP certified.