

**The Effects of Sewage Discharge on The Marine Gastropod *Gibbula* sp.,
Collected From The Coast of Al-Hanyaa, Libya.**

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ABSTRACT

Marine gastropods are known to accumulate high metal concentrations in their tissues especially in the digestive glands. In this study, the accumulation of Cu, Zn, Cd, Cr and Pb in the gastropod *Gibbula* sp. (Family: Trochidae) has been assessed using Atomic absorption, spectrophotometry graphite Furnace. Animals were collected from rocky shores at Al-Hanyaa region on the north-east coast of Libya. Two groups of animals were studied; one from an area adjacent to a sewage outlet and the second was collected from a relatively clean site. Concentrations of metals in the viscera, of both adults and juveniles, of *Gibbula* collected from the polluted site were higher than that recorded in the clean site. Thus, there is a tendency for *Gibbula* tissues in the polluted area to have higher concentrations of metals although the differences are not always statistically significant. The values recorded for adult specimens collected from the polluted and non polluted locations were 0.51 ± 0.27 , 15.95 ± 1.28 , 0.021 ± 0.017 , 0.47 ± 0.18 , 0.60 ± 0.17 vs 0.40 ± 0.11 , 9.51 ± 4.82 , 0.03 ± 0.02 , 0.29 ± 0.09 , 0.39 ± 0.09 for Cu, Zn, Cd, Cr and Pb $\mu\text{g}/\text{mg}$, respectively. Whereas, those recorded for juveniles were 0.51 ± 0.09 , 17.02 ± 1.13 , 0.032 ± 0.01 , 0.49 ± 0.15 , 0.54 ± 0.17 vs 0.38 ± 0.22 , 8.36 ± 2.05 , 0.020 ± 0.01 , 0.35 ± 0.25 , 0.52 ± 0.12 for Cu, Zn, Cd, Cr and Pb $\mu\text{g}/\text{mg}$, respectively. It is suggested that metals are taken up by *Gibbula* sp. viscera directly from the water or from the ingested algae. Also, the shell mass was affected with the pollution.

Keywords: Bioindicators; Heavy metals, Mediterranean sea, *Gibbula* sp.

INTRODUCTION

Heavy metals are among the most common environmental pollutants, and their occurrence in waters and biota indicate the presence of natural or anthropogenic sources associated with industrial and domestic effluents (Biney *et al.*, 1994; Zarazua *et al.*, 2006). They can be easily accumulated by the primary consumer (algae), and biomagnified through their transmission across the higher levels of the food chain causing a direct effect on the human health (Page, *et al.*, 1987). Lead, copper, cadmium, Chromium and arsenic were reported as the most toxic heavy metal pollutants (Hutton, 1987). The establishment of an efficient monitoring program to identify the contaminant in a given ecosystem became an important priority (Abd-Allah and Bream, 2001).

Marine gastropods are known to accumulate high metal concentrations in their tissues, especially in the digestive glands. *Gibbula* is a genus of small sea snails, known as top snails or top shells, marine gastropod molluscs in the family Trochidae inhabiting the upper intertidal zone on rocky shores, where they graze on algal films

or macroscopic algae. Many of them are very tolerant to exposure, living around the high tide mark with only brief periods of immersion.

Sewage effluent is often discharged via outfall into shallow subtidal habitats. Along the coasts of the Mediterranean Sea, despite a large amount of domestic and industrial sewage is discharged to the sea (USEPA, 2003), few published account exists concerning the effects of sewage effluents on gastropods inhabiting rocky shores. On rocky shores, sewage discharges can modify natural distribution patterns of sessile organisms. The impact of sewage on shallow hard substrate assemblages has been assessed along SW Apulian coast, Italy, (Terlizzi *et al.*, 2002). The present study aimed to investigate the effects of a sewage discharge in Al Hanyaa city coastal region on the ecosystem using marine gastropods as biological monitors to prevent pollution wherever possible and to reduce risk for people and ecosystems.

MATERIALS AND METHODS

Collecting of Samples

The gastropod, *Gibbula* sp., was examined for heavy metal (Cu, Zn, Pb, Cd, and Cr) levels in their soft parts. Samples were collected by hand from polluted and unpolluted sites located on the coast of Al-Hanyaa during fall 2009. Immediately after collection, snails were transferred fresh inside plastic containers to the laboratory. In the laboratory, all samples were washed with running distilled water. Prior to analysis, samples were classified, weighed and measured and the whole soft tissues of both adults and juveniles were removed from their shells and prepared for analysis. Specimen size was ranging between 1.2 and 1.5 cm shell length and 1.8-2.2 cm shell width, while the weight ranged between 5.0 – 6.2 gm throughout the work to reduce possible variations in metal concentrations due to size and age (NAS, 1980) and to assure sampling of mature specimens. In addition, the impact of the outfall on juvenile *Gibbula* was also tested.

The soft tissues were weighed and wet digested in concentrated nitric acid and heavy metals levels have been determined in the digested tissues, and water samples using graphite furnace atomic absorption spectroscopy instrument (Perkin Elmer) based on the method of McDaniel, 1991, and Kraak, *et al.*, 1993. The concentration was calculated in µg/mg fresh weight.

Observed bioaccumulation factors (BAFs) of these contaminants were calculated according to Harrahy and Clements (1997) using the following formula:

$$BAF = C_t/C_w$$

Where

C_t = tissue concentration

C_w = water concentration

Statistical analysis

Student's t-test was used to compare least square of means of metal concentration in different sites.

RESULTS AND DISCUSSION

The specimens of gastropods were sampled from two sites. The two locations were approximately 3–4 km from each other and situated on rocky shore. One considered polluted, located directly on the outfall (outlet of a sewage treatment station), the other used as a reference area “clean or unpolluted”. The outfall serves more than 30,000 inhabitants. Waste waters are supposed to be biologically and

chemically treated, by biwater company, before discharging. Trace metal concentrations in snails collected in September 2009 from these locations of Al-Hanyaa coast are presented in Tables 1-3 and plotted in Figs. 1-3. The results of specimens collected from polluted site showed that most metal concentrations in soft tissues of snails (adult and juvenile) were higher than that recorded in the clean site. Thus, there is a tendency for *Gibbula* tissues in the polluted area to had higher concentrations for all metals compared to clean area, although the differences are not always statistically significant. The attained results agree with those of Nicolaidou, and Nott, 1998 who stated that the concentrations of metals in the viscera of the gastropods *Cerithium vulgatum* and *Monodonta mutabilis* are higher than in the muscle and significantly higher in the animals from the polluted site. The same authors studied the uptake of metals by some gastropods, the accumulation sites and the release of the heavy metals in gastropods and found that these factors depend on the genera and the sites. Their findings proved that metals are accumulated within intracellular mineralized granules as phosphates and within lysosomal residual bodies in association with sulphur (Nott and Nicolaidou, 1989, 1990, 1994).

The highest accumulated metals were Zn and Cu, while the lowest one was Cd in both adult and juvenile stage. The values recorded for adult specimens collected from the polluted and unpolluted locations were 0.51 ± 0.27 , 15.95 ± 1.28 , 0.021 ± 0.017 , 0.47 ± 0.18 , 0.60 ± 0.17 vs 0.40 ± 0.11 , 9.51 ± 4.82 , 0.03 ± 0.02 , 0.29 ± 0.09 , 0.39 ± 0.09 for Cu, Zn, Cd, Cr and Pb $\mu\text{g}/\text{mg}$, respectively. Similar results were recorded for juveniles, 0.51 ± 0.09 , 17.02 ± 1.13 , 0.032 ± 0.01 , 0.49 ± 0.15 , 0.54 ± 0.17 vs. 0.38 ± 0.22 , 8.36 ± 2.05 , 0.020 ± 0.01 , 0.35 ± 0.25 , 0.52 ± 0.12 for Cu, Zn, Cd, Cr and Pb $\mu\text{g}/\text{mg}$, respectively. It is suggested that metals are taken up by *Gibbula* sp. viscera directly from the water or from the ingested algae. Depending on the values of bioaccumulation factors, the relative bioconcentration variability of the studied dissolved metals in adults followed the order: $\text{Pb} > \text{Zn} > \text{Cu} > \text{Cr} > \text{Cd}$ where the juvenile followed the order: $\text{Pb} > \text{Cu} > \text{Cr} > \text{Zn} > \text{Cd}$. Similar results were obtained by Catsiki and Florou (2006) in the Saronikos and Thermaikos Gulf (Greece) with *Mytilus galloprovincialis*.

Also, the shell mass was affected with the pollution where it is noticed that the shells of snails collected from the polluted sites were more fragile than those collected from the reference site. The agreed with that mentioned by Beeby *et al.*, 2002, who found that Pb, affected the shell growth of laboratory-bred offspring of *Helix aspersa*.

Further studies of metal levels in various other intertidal organisms from Al-Hanyaa rocky shore and the Libyan eastern coast, together with investigations into the possible effects of seasonal changes on metal concentration and distribution, are currently under way.

Table (1): Heavy metals concentrations (mg/L) determined in water samples collected from the rocky shore of Al-Hanyaa, Libya.

Location/Metal	Cu	Zn	Cd	Cr	Pb
Polluted	0.6	7.4	0.07	0.32	0.4
Unpolluted	0.24	5.4	0.03	0.22	0.2

Table 2: Heavy metals concentrations ($\mu\text{g}/\text{mg}$ wet weight) determined in *Gibbula* adults collected from two sites of the rocky shore of Al-Hanyaa, Libya.

Location/Metal	Cu	Zn	Cd	Cr	Pb
Polluted	$0.51\pm 0.27\text{a}$	$15.95\pm 1.28\text{a}$	$0.021\pm 0.017\text{a}$	$0.47\pm 0.18\text{a}$	$0.60\pm 0.17\text{a}$
BAFs	1.0	2.2	0.3	1.2	1.5
Unpolluted	$0.40\pm 0.11\text{a}$	$9.51\pm 4.82\text{c}$	$0.03\pm 0.02\text{a}$	$0.29\pm 0.09\text{b}$	$0.39\pm 0.09\text{c}$
BAFs	1.7	1.8	1.0	1.3	2.0

Means followed with the same letter are not significantly different.

Table 3: A comparison of heavy metal concentrations ($\mu\text{g/g}$ wet weight) determined in Juvenile individuals of *Gibbula* sp. collected from polluted and unpolluted sites of Al-Hanyaa, Libya.

Location/Metal	Cu	Zn	Cd	Cr	Pb
Polluted	$0.51 \pm 0.09\text{a}$	$17.02 \pm 1.13\text{a}$	$0.32 \pm 0.01\text{a}$	$0.49 \pm 0.15\text{a}$	$0.54 \pm 0.17\text{a}$
BAFs	0.9	2.3	4.6	1.5	1.4
Unpolluted	$0.38 \pm 0.22\text{a}$	$8.36 \pm 2.05\text{c}$	$0.020 \pm 0.01\text{a}$	$0.35 \pm 0.25\text{a}$	$0.52 \pm 0.12\text{a}$
BAFs	1.6	1.5	0.7	1.6	2.6

Means followed with the same letter are not significantly different.

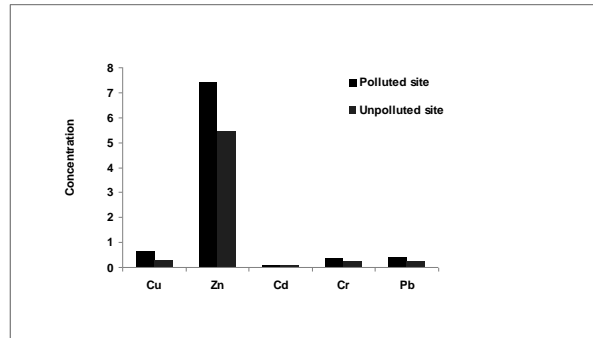


Fig. 1: Heavy metals concentrations (mg/l) determined in water samples collected from the rocky shore of Al-Hanyaa, Libya.

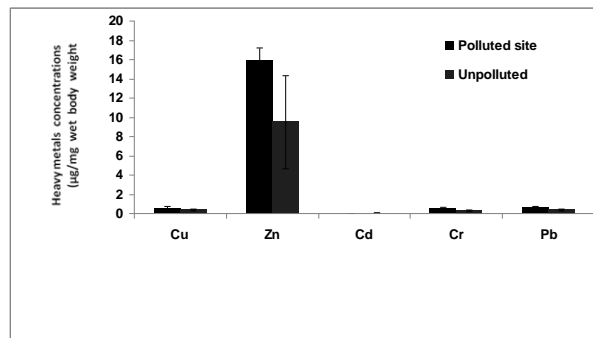


Fig. 2: A comparison of heavy metal concentrations ($\mu\text{g/mg}$ wet body weight) determined in adults and Juvenile individuals of *Gibbula* sp. collected from Al-Hanyaa coast.

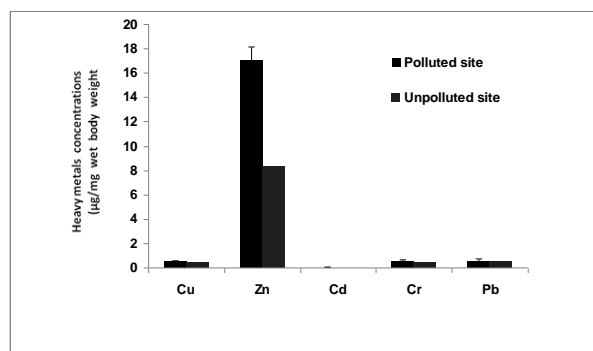


Fig. 3: A comparison of heavy metal concentrations ($\mu\text{g/mg}$ wet body weight) determined in adults and Juvenile individuals of *Gibbula* sp. collected from Al-Hanyaa coast.

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ARABIC SUMMARY

تأثيرات صرف مياه المجاري على البطنقديات البحرية من نوع جيببولا المجمععة من ساحل الحنية، ليبيا

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معروف عن البطنقديات البحرية أنها تجمع تركيزات عالية من العناصر في أنسجتها، خاصة في الغدد الهضمية. في هذه الدراسة، تم تعيين تراكم كل من النحاس، الزنك، الكاديوم، الكروم والرصاص في البطنقدي من نوع جيببولا (فصيلة: تروكيدي) باستخدام جهاز الامتصاص الذري ذو فرن الجرافيت لقياس شدة موجات الطيف. جُمعت الحيوانات من الشواطئ الصخرية في منطقة الحنية على الساحل الشمالي الشرقي لليبييا. تم دراسة مجموعتين من الحيوانات؛ واحدة من منطقة مجاورة لمخرج مياه مجاري والثانية من موقع نظيف نسبياً. وكانت تركيزات المعادن في أحشاء الطور البالغ وطور الشباب المجمععة من الموقع الملوّث أعلى من تلك التي سجّلت في الموقع النظيف.

هكذا، كان هناك ميل لأنسجة الجيببولا في المنطقة الملوّثة لتجميع تركيزات أعلى من المعادن بالرغم من أن هذه الزيادة ليست معنوية إحصائياً دائماً. وكانت القيم المسجلة للعينات البالغة التي جُمعت من المواقع الملوّثة وغير الملوّثة كالتالي: 0.27 ± 0.51 , 1.28 ± 15.95 , 0.017 ± 0.021 , 0.18 ± 0.47 , 0.17 ± 0.60 مقابل 0.11 ± 0.40 , 4.82 ± 9.51 , 0.02 ± 0.03 , 0.09 ± 0.29 , 0.09 ± 0.39 ميكروجرام/مليجرام من وزن الحيوان لكل من النحاس، الزنك، الكاديوم، الكروم و الرصاص على التوالي. بينما تلك المسجلة للأحداث 0.09 ± 0.51 , 1.13 ± 17.02 , 0.01 ± 0.032 , 0.15 ± 0.49 , 0.54 ± 0.17 مقابل 0.22 ± 0.38 , 2.05 ± 8.36 , 0.01 ± 0.020 , 0.25 ± 0.35 , 0.12 ± 0.52 ميكروجرام / مليجرام لكل من النحاس، الزنك، الكاديوم، الكروم و الرصاص على التوالي. ويُعتقد أنّ المعادن تؤخذ من قبل الأحشاء مباشرة من الماء أو من الطحالب المُبتلعة. أيضاً، كما لوحظ أن كتلة الصدفة قد تأثرت بالتلوث. كلمات دالة: دليل حيوي، العناصر الثقيلة، البحر المتوسط، قوقع الجيببولا.