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Microbiological pollution and heavy metals in two freshwater turtles from Garmat Ali- River in Basrah City/ Southern Iraq

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Abstract:

The microbiological pollution and some heavy metals (Fe, Cu, Zn, Al and Pb) levels in two freshwater turtles species (*Mauremys caspica* (Gmelin 1774), and *Rafetus (Trionyx) euphraticus* (Daudin 1802)) collected from Garmat Ali- River. Samples of two freshwater Turtles were collected during summer season of the year 2017, were examined. Total- plate techniques were used for microbial pollution while heavy metal concentrations in two turtles species were determined using atomic absorption spectrophotometer (AAS). Total bacteria count had the highest number of bacteria in *M. c. caspica* with 29.60×10^5 cfu/ml. the total plate count had the highest bacteria number in the *R. euphraticus*– 19.26×10^5 cfu/ml ranged. bacterial pathogens isolated include: *E. coli*, *Pseudomonas spp.*, *Vibrio spp.*, and *Staphylococcus spp.* Microbial species were characterized based on morphological and biochemical tests. *M. c. caspica* Turtle had iron concentration of 21.52 ± 0.031 mg/kg while *R. euphraticus* Turtle had iron concentration of 34.69 ± 0.152 mg/kg. *M. c. caspica* and *R. euphraticus* had copper concentrations of 0.87 ± 0.034 and 0.92 ± 0.057 mg/kg respectively. *M. c. caspica* had a lead concentration of 0.06 ± 0.041 mg/kg while *R. euphraticus* had a copper concentration of 0.09 ± 0.064 mg/kg.

Keywords: Microbiological pollution, heavy metals, *E. coli*, bacterial pathogens, *Mauremys c. caspica* turtles.

Introduction

Three species of freshwater turtles and tortoise are recorded from Iraq: the Mediterranean spur-thighed tortoise, *Testudo graeca terrestris* (Forskall 1775), Eastern Caspian turtle, *Mauremys c. caspica* (Gmelin 1774), and Euphrates soft-shelled turtle *Rafetus (Trionyx) euphraticus* (Daudin 1802), [1] (Al-Barwari and Saeed 2007). Both species, *R. euphraticus* and *M. c. caspica*, are probably threatened in Iraq. The turtles of Iraq have received scant scientific attention since Khalaf (1959); however, little is known about their distribution and status [2, 3, 4].

The Garmat Ali River was exposed to multiple impacts of hydrological and human activities. After inundation of the southern marshes in 2003, the East Hammar marsh was fed primarily from the Euphrates River and entering the Garmat Ali River then the Shatt Al-Arab River that eventually flows into the Arabian Gulf. So, this river affected by the water from the Euphrates and tidal current of the Gulf through the Shatt Al-Arab River. But, the flow of the Euphrates was diverted away from the north East Hammar marsh during the last years, consequently the water level in the marsh dropped sharply, causing the water salinity higher than before, which led to negatively affect on water quality and quantity of the Garmat Ali River [5]. Moreover, the Shatt Al-Arab River suffered from massive regression in water quality related to the decline in rates of discharge from the Tigris and the Euphrates Rivers [6] as a result of several hydrological projects constructed in the riparian countries [7], and the diversion of the Karun River into Iranian terrene [8].

Microbial and heavy metals from polluted industrial sewage may contaminate the turtle feeding grounds. Also other studies in Iraq reported Microbial contamination and heavy metal contamination in fish and other organisms from Garmat Ali River and Shatt Al-Arab river [9, 10, 11, 12].

The objectives of this study are to determine microbial contamination and qualities in skin, intestine content and muscle tissues of *Glemmyscaspica* Turtles in Garmat Ali- River. This study is crucial and may be used as a conservation indicator for the survival and tolerance of freshwater turtles in this region.

Material and Methods

Sample collection and identification

Garmat Ali River, Basrah city, south of Iraq. It is a waterway between the East Hammar marsh and the Shatt Al-Arab River (Fig. 1). It is about 6 km, 280 m width and the mean depth is 9 m. The river is affected by the tidal current of the Arabian Gulf through the Shatt Al-Arab River. Samples were collected from the one site on along the river, near from Basrah university site in Garmat Ali River during summer 2017. Samples were collected at a depth of 20 – 100 cm. The predominant vegetations on the banks were *Phragmites australis*, and *Typhadomingensis*, whereas *Ceratophyllum demersum* and *Potamogeton crispus* were dominant in the deeper areas.

A total of (20) fresh water turtles were collected randomly by hand net captured. Were collected aseptically and immediately transported in a thermal bag to the laboratory and processed within 3hrs of acquisition.

The bacterial counts on the external surfaces were estimated as follows:

Skin surfaces: Sample from some locations of the skin of 20 life turtles was taken by rubbing the sterilized cotton swab over the skin and then inoculated into 9ml of Nutrient broth, MacConkey broth and Selenite F broth which are dispensed in separate tubes. 10 fold serial dilution of the bacterial suspension already inoculated in peptone water was prepared in

duplicate and viable aerobic bacterial counts were enumerated using 0.1ml and 1ml inoculums in standard plate count agar as described by Slabyet *al.*, (1981) [13], and then incubated at 37°C for 48 hrs.

Total bacteria counts were determined using pour plate method with MacConkey agar, EMB Agar respectively. Mueller-Hinton Agar for *Pseudomonas spp.* *Salmonella spp.* and *Shigella spp.*, were enumerated using Salmonella Shigella Agar (SSA) and Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar for pathogenic *Vibrio spp.* The plates were incubated at 35±2°C for 24h. The observed colony growth was counted using Coulter™ Colony counter according to plate count method. Identification of the organisms was done using the phenotypic and biochemical characteristics.

Heavy Metal Analysis: The turtles skin samples were homogenized separately in a mortar and weighed accurately in a porcelain crucible. Before ashing, 1ml of concentrated HNO₃ was added to the samples and allowed to pre-digest overnight in order to reduce losses of volatile metals. The samples were charred on an electric hot plate before ashing in a muffle furnace at 550°C for 4hrs. The white ash was dissolved in 5 ml of 1:1 HCl and a solution made in a 50 ml standard flask [14]. Metal concentrations of the samples were read against appropriate blank and standard solutions using a Perkin-Elmer model 306 Atomic Absorption spectrophotometer (AAS). A blank solution for the biotic samples was made by diluting 1ml concentrated HNO₃ with 5ml 1:1HCl and a 50ml solutions made up with distilled water. The individual metals were expressed on a dry weight basis as mg/kg and the data generated were analyzed statistically.

Statistical Analysis

The statistical analysis of the tabulated data was performed by using SPSS (18.0 version) to compare the significant difference between means at $p < 0.05$.

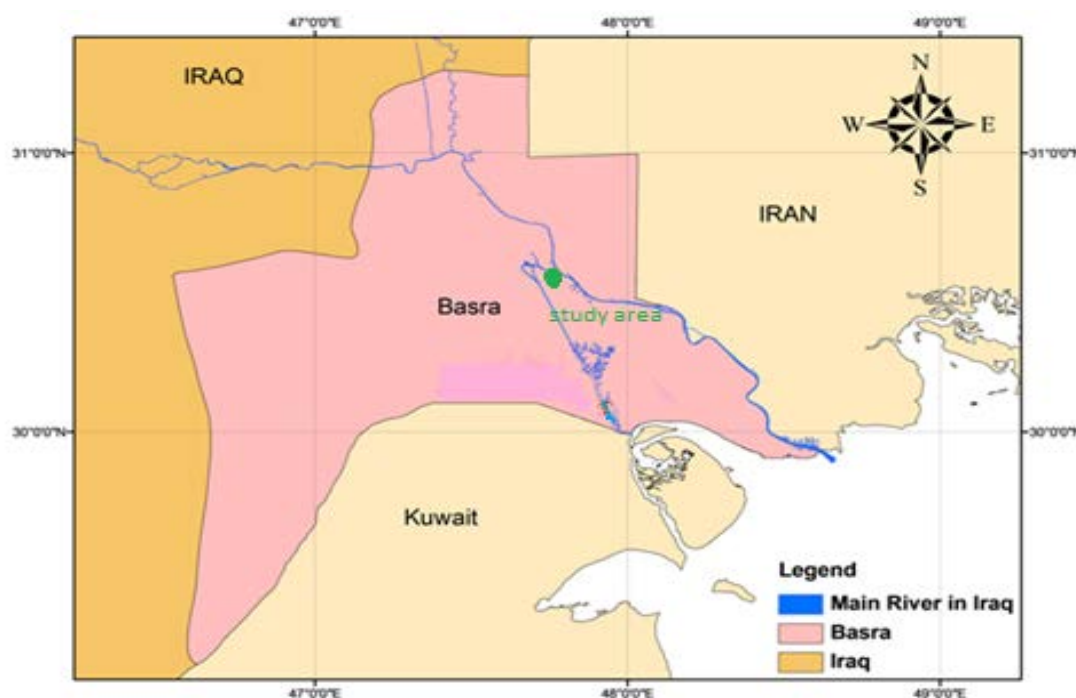


Fig. 1: map of study area

Results

In this study, the total plate count (TPC) of the 20 turtles samples of two Turtles species was analysed in skin. The highest number of bacteria in *M. c. caspica* was 29.60×10^5 cfu/ml. The total plate count had the highest number in the *R. euphraticus* – 19.26×10^5 cfu/ml showed in Table 1; revealed the isolation of *Pseudomonas* spp. In the skin of *M. c. caspica* having the highest number in (53.73×10^3 cfu/ml), while in the *R. euphraticus* skin had the highest number of *Pseudomonas* isolates. Isolated had the total count of 37.07×10^3 cfu/ml from the *R. euphraticus* samples. No isolation of *Vibrio* spp. on the *M. c. caspica* skin while *R. euphraticus* skin had a high count of 7.19×10^2 cfu/ml (Table 1). *E. coli* isolation showed the highest count in skin of the *M. c. caspica* 85.45×10^4 while *R. euphraticus* skin (26.18×10^4 cfu/ml). The *Staphylococcus* spp. had a low isolation rate in all samples of two species were analysed as generally compared with other isolated from other organisms except *Vibrio* spp. that had the lowest (Table 1). *Salmonella* spp. and *Shigella* spp. are not isolated in all turtle's samples analysed in this study.

Table. 1: The total number of bacteria count cfu/ml in skin of two Turtles species.

Turtles species	TPC Cfu/ml	Pseudomonas spp. Cfu/ml	E. coli Cfu/ml	Staphylococcus spp. Cfu/ml	Vibrio spp Cfu/ml	S/S Cfu/ml
<i>M. c. caspica</i>	29.60×10^5	53.73×10^3	85.45×10^4	4.46×10^2	NI	NI
<i>R. euphraticus</i>	19.26×10^5	37.07×10^3	26.18×10^4	3.15×10^2	1.19×10^2	NI

Where: TPC- Total Plate Count, S/S – *Salmonella* spp./*Shigella* spp., NI – No isolation

All bacterial isolates including pathogens and antagonistic isolates were identified using biochemical and morphological tests. The species of bacteria isolated were identified and predominant by gram-negative bacteria. Of these, (*Escherichia coli*, *Pseudomonas* spp., *Vibrio* spp. and *Staphylococcus aureus*). Were found common in skin of two species Turtles. Table (2). *Escherichia coli* had the highest frequency of 90% while *Streptococcus* spp. and *Vibrio* spp. had a highest frequency of 10% respectively. Table (3).

Table. 2: Morphological and Biochemical characteristics of bacteria species isolated from sampling of the skin of two species Turtles.

bacteria species. Reaction agent	<i>Pseudomonas</i> <i>spp.</i>	<i>Escherichia coli</i>	<i>Vibrio spp.</i>	<i>Staphylococcus</i> <i>spp.</i>
Gram staining	G ^{-ve} bacilli	G ^{-ve} bacilli	G ^{-ve} bacilli	G ^{+ve} cocci
Shape	M	M	M	Ma
Motility	+	-	-	-
Indole test	+	-	-	-
Methyl red test	-	-	-	-
Vogesproskuer test	-	+	-	+
Citrate utilization test	-	+	+	+
Urease test	-	A/A+G	A/A	K/K
TSI test	-	-	+	-
H ₂ S	-	+	-	+
Gas	+	-	-	-
Nitrate reduction test	+	+	+	+
Catalase test	+	+	-	+
Oxidase test	+	+	-	+
Carbohydrate test	-	-	-	-
Glucose	-	-	-	-
Maltose	-	-	-	-
Sucrose	-	-	-	-

Note: - Negative, + Positive, A/A -Glucose& lactose and /or sucrose fermentation, K/K -no fermentation, A/A+G- Glucose& lactose and /or sucrose fermentation+ gas produced.

Table. 3: Frequency distribution of bacteria isolates from skin samples of two species Turtles

Bacteria species isolate	Percentage frequency (100%)
<i>Escherichia coli</i>	90
<i>Pseudomonas spp.</i>	76
<i>Vibrio spp.</i>	10
<i>Staphylococcus spp.</i>	10

Table. 4 shows the levels of Fe, Cu and Pb that were expressed in mg/kg dry weight sample. The data clearly shows variation in the level of heavy metals among the freshwater turtles species sampled.

The analysis of the selected metals in the present study revealed an order of Fe>Cu>Pb in two freshwater turtles species.

M. c. caspica Turtle had an iron concentration of 21.52 ± 0.031 mg/kg while *R. euphraticus* Turtle had an iron concentration of 34.69 ± 0.152 mg/kg. *M. c. caspica* and *R. euphraticus* had copper concentrations of 0.87 ± 0.034 and 0.92 ± 0.057 mg/kg respectively. *M. c. caspica* had a lead concentration of 0.06 ± 0.041 mg/kg while *R. euphraticus* had a copper concentration of 0.09 ± 0.064 mg/kg.

Table. 4: Heavy metals concentration (mg/kg) in two Turtles species

Turtles species	Fe	Cu	Pb
<i>M. c. caspica</i>	21.52 ± 0.031	0.87 ± 0.034	0.06 ± 0.041
<i>R. euphraticus</i>	34.69 ± 0.152	0.92 ± 0.057	0.09 ± 0.064

Discussion

Specimens of *M. c. caspica* were collected from the Garmat Ali River because this station that good conditions in comparison with the other sites in Shatt Al-Arab river.

Garmat Ali station has alluvial soil that is suitable for *M. c. caspica* nesting [15] (Kami *et al.* 2006) added to calm waters and low salinity rate of their tributaries which created a favorable conditions for turtles [4] (Ghaffari *et al.* 2008).

Continuous exposure of organisms, such as freshwater turtles, to heavy metals resulted in chronic intoxication which may be fatal [16, 17] (Harper *et al.* 2007; Storelli *et al.* 2008).

heavy metal deposition from industrial activities, aquaculture activities, treated and untreated municipal wastewater from urban developments may lead to heavy metals accumulation in the feeding habitats. [18, 19] (Al-Musharaf *et al.* 2014a; 2014b). Fe is an essential element in human diet and Iron forms part of hemoglobin which allows oxygen to be carried from the lungs to the tissues.

In this study, The copper concentrations were similar to other studies of [20, 21, 22] (Yilmaz, 2009; Rejomon *et al.* 2010; Olowu *et al.* 2010).

In this study, Higher concentration of lead is known to inhibit active transport mechanisms involving ATP and may also suppress cellular oxidation-reduction reactions and even inhibit of protein synthesis (Adeyeye *et al.* 1996). The level of lead from this study, could not be said to pose any health risk since the values were within the FAO permissible limit of 0.3 mg/kg.

In addition to heavy metals, several studies related to pathogenic bacteria were published, in Iraq some studies revealed that sewage contaminated effluents contribute to environmental pollution (Abed *et al.* 2016; Alkanany *et al.* 2017; Al-Khafaji *et al.*, 2018) [9, 10, 12].

In parts of the world, including Iraq, pathogenic bacteria were isolated from turtles, fish and fresh water habitats.

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