

Accumulation of Heavy Metals in Tilapia Fish (*Oreochromis niloticus*) from Al-Khadoud Spring, Al-Hassa, Saudi Arabia

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Abstract: Problem statement: Al-Khadoud Spring is one of the largest spring in Al-Hassa governorate (Saudi Arabia). Due to the extended domestic activities and urbanization as well as the continuous industrial and agricultural growth of the region, spring water quality is potentially changing. **Approach:** This study was conducted to measure toxic heavy metal concentrations in water and fish samples along the spring channel. Filtered spring water and tissues (liver and muscle) of captured fish were analyzed for heavy metals in an Atomic Absorption Spectrophotometer equipped with a Varian Model. **Results:** The concentrations of metals in water were found in the following order: $Fe^{2+} > Zn^{2+} > Cu^{2+} > Pb^{2+} > Mn^{2+} > Cd^{2+}$. The levels of heavy metals recorded in water in this study were generally low, when compared to WHO and USEPA recommended levels in water, except iron which was found to be higher than the recommended levels. In fish samples collected from the polluted spring, metal levels were significantly higher than the levels in water, indicating bioaccumulation. The highest levels of metals were generally reported in fish liver than muscle. Hepatic metal levels were ranked as follow: $Zn^{2+} > Cu^{2+} > Pb^{2+} > Cd^{2+}$. **Conclusion:** The present results showed that, the fishes, based on the higher levels of metal bioaccumulation, could be unsafe for human consumption. Consequently, very close monitoring of heavy metal loads in Al-Khadoud spring is recommended in view of the possible risks to health of consumers.

Key words: Al-Khadoud spring, Al-Hassa, water pollution, heavy metals, *Oreochromis niloticus*, bioaccumulation

INTRODUCTION

Chemicals derived from agricultural operations (pesticides and herbicides) and industrial effluents, such as metals, ultimately find their way into a variety of different water bodies and can produce a range of toxic effects in aquatic organisms, ranging from alterations to a single cell, up to changes in whole populations^[1]. The accumulation of toxic metals to hazardous levels in aquatic biota has become a problem of increasing concern. Excessive pollution of surface waters could lead to health hazards in man, either through drinking of water and/or consumption of fish^[2]. The increasing importance of fish as a source of protein and the interest in understanding the accumulation of heavy metals at the trophic levels of the food chain, extend the focus towards finfish^[3,4]. Pollution enters fish through five main routes: Via food or non-food particles, gills, oral consumption of water and the skin. On absorption, the pollutant is carried in blood stream to either a storage point or to the liver for transformation and/or storage. Pollutants transformed in the liver may be stored there or excreted in bile or transported to other excretory organs such as gills or kidneys for elimination or stored in fat,

which is an extra hepatic tissue^[5,6]. The concentration of any pollutant in any given tissue therefore depends on its rate of absorption and the dynamic processes associated with its elimination by the fish.

Stocks of both freshwater and marine fish within Saudi Arabia are increasingly threatened by aquatic pollution, but no data are available on the extent pollution impacts. Our previous study at El-Khadoud spring showed that its water had an obvious increase in electrical conductivity, Chemical Oxygen Demand (COD), total alkalinity, nitrates, phosphorus, chloride and potassium^[7]. These features indicated pollution with organic wastes, increased salinity and deteriorated oxygenated state. In the present work, heavy metal accumulation levels in water samples and fish tissues (liver and muscle) of *Oreochromis niloticus* from that contaminated water body at Al-Hassa were determined to assess the public health risks associated with consuming fish harvested from this area.

MATERIALS AND METHODS

Study area: Al-Khadoud Spring is one of the largest spring in Al-Hassa governorate. Al-Hassa is situated at 25°05' and 25°40' northern latitude and 49°55' eastern

longitude; located in eastern region of Saudi Arabia, 140 km south-west of Dammam city (Fig. 1). Al-Hassa has a dry, tropical climate, with a five-month summer and a relatively cold winter. This oasis region is well-known for its date farming. To increase water for irrigation, the water pumped into the spring canals so to increase the amount of water used for local irrigation. Over the years, the spring and its connected canals have become a place of fish and other aquatic organisms' growth. In fact, this water body has attracted the attention of local people for fishery. Due to the extended domestic activities and urbanization as well as the continuous industrial and agricultural growth of the region, spring water quality is potentially changing. Some of untreated domestic and agricultural sewage may elapse into spring water. Such contamination may be an important issue regarding the health of the aquatic ecosystem and its animals and in turn, to human's health.

Sampling strategy: Tilapia fish (*Oreochromis niloticus*) with length of 12-19 cm and weight of 40-60 g were collected by bottom trap net from Al-Khadoud spring channel. Sampling was carried out monthly between January and December 2007. Fish samples were taken from three stations. Station I is the main water basin of Al-Khadoud spring. It is located between 25°N and 49°E. The water at this point is relatively unpolluted. Stations II and III are located on the irrigational channel of the spring (5 and 10 km north of Al-Khadoud spring, respectively) (Fig. 2). Station III is a point source of effluents (sewage and runoff from agricultural fields). Fishing is intense at this station. On each occasion, fish samples were collected in three replicate spots at each station. Fish samples were placed in plastic bags and were stored frozen at -10°C after cleaning with distilled water to remove adhering dirt. Spontaneously with fish capture, sampling of water was carried out from the particular catch area. Water samples were taken at 20 cm depth in 250 mL plastic bottles cleaned with detergent and soaked overnight in 5% nitric acid. All samples were stored frozen at -10°C.

Sample treatment: All frozen samples were allowed to thaw at room temperature. Water samples were mixed vigorously and aspirated in an Atomic Absorption Spectrophotometer for trace metal determination^[8]. The fish samples after defrosting were dissected; liver and muscle were taken with the help of a stainless steel stiletto. After dissection, all the tissue samples were separately oven-dried to constant weight at 105±20°C and were each ground to powder. The powdered samples were digested according to Sreedevi *et al.*^[9].



Fig. 1: Map of the study area

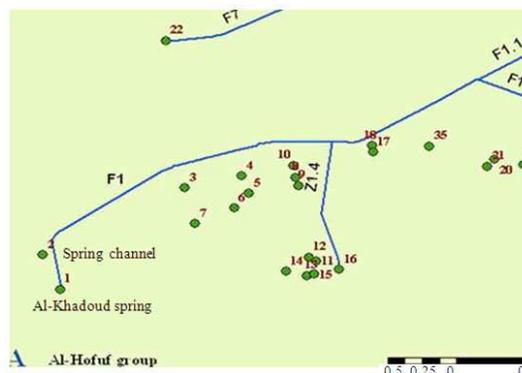


Fig. 2: Map of Al-Khadoud spring and other water springs in Al-Hassa Oasis

One gram of each sample was digested using 1.5.1 mixture of 70% perchloric acid, concentrated nitric acid and concentrated sulphuric acid at 80±5°C in a fume chamber, until colorless liquid was obtained. Each digested sample was made up to 20 mL with de-ionized water and analyzed for heavy metals in an Atomic Absorption Spectrophotometer (AA-6800F, Shimadzu, Japan) equipped with a Varian Model (GFA-EX7, Shimadzu, Japan)^[8]. Values of heavy metals were recorded in µg g⁻¹ dry weight.

Statistical analysis: One-way ANOVA was used for detection of differences in heavy metal concentrations between locations. Individual differences were detected by appropriate ad hoc tests (LSD). p<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The process whereby an organism concentrates metals in its body from the surrounding medium or food, either by absorption or ingestion is known as bioaccumulation^[10]. According to Heath^[5], fish can regulate metal concentration to a certain limit after which bioaccumulation occurs. The concentration of metals in an organism's body vary from organ to organ and is the product of an equilibrium between the concentration of the metal in an organism's environment and its rate of ingestion and excretion^[11-13].

Table 1: Mean values of some tested heavy metals concentrations (mg L⁻¹) in water of Al-Khadoud spring channel compared to the international permissible limits

Metal	Cd ²⁺	Cu ²⁺	Fe ²⁺	Mn ²⁺	Pb ²⁺	Zn ²⁺
Station I	0.004 ^a	0.005 ^a	0.980 ^a	ND	ND	0.030 ^a
Station II	0.004 ^a	0.005 ^a	0.982 ^a	ND	ND	0.030 ^a
Station III	0.004 ^a	0.022 ^b	2.210 ^b	0.017	0.021	0.031 ^a
WHO ^[15]	0.05	1.000	1.000	0.010	0.050	5.000
USEPA ^[16]	0.01	1.000	1.000	0.050	0.050	1.000

Means (n = 10); ND: Non Detectable; Values within columns with no common superscripts are significantly different (p<0.05)

Table 2: Seasonal copper concentrations (Cu²⁺) (µg g⁻¹ dry weight) in organs of Tilapia fish, *Oreochromis niloticus*, collected from Al-Khadoud spring channel at station III during the investigation period

Seasons	Liver	Muscles
Autumn	216.23±28.50 ^a	1.73±0.16 ^a
Winter	146.02±7.05 ^b	2.53±0.20 ^a
Spring	644.96±58.90 ^c	2.52±0.17 ^a
Summer	445.16±18.33 ^d	3.06±0.20 ^a
Total average	363.09	2.46

Means ± SE (n = 10); Values within columns with no common superscripts are significantly different (p<0.05)

Table 3: Seasonal cadmium concentrations (Cd²⁺) (µg g⁻¹ dry weight) in organs of Tilapia fish, *Oreochromis niloticus*, collected from Al-Khadoud spring channel at station III during the investigation period

Seasons	Liver	Muscles
Autumn	17.65±1.76 ^a	0.39±0.04 ^a
Winter	12.73±0.39 ^a	0.19±0.03 ^a
Spring	3.13±0.29 ^b	0.25±0.02 ^a
Summer	2.66±0.14 ^b	0.31±0.03 ^a
Total average	9.04	0.28

Means ± SE (n = 10); Values within columns with no common superscripts are significantly different (p<0.05)

Table 4: Seasonal lead concentrations (Pb²⁺) (µg g⁻¹ dry weight) in organs of Tilapia fish, *Oreochromis niloticus*, collected from Al-Khadoud spring channel at station III during the investigation period

Seasons	Liver	Muscles
Autumn	133.06±11.95 ^a	1.65±0.07 ^a
Winter	119.03±1.47 ^a	1.51±0.07 ^a
Spring	113.12±1.25 ^a	1.38±0.03 ^a
Summer	47.86±0.81 ^b	1.41±0.04 ^a
Total average	103.26	1.49

Means ± SE (n = 10); Values within columns with no common superscripts are significantly different (p<0.05)

Table 5: Seasonal zinc concentrations (Zn²⁺) (µg g⁻¹ dry weight) in organs of Tilapia fish, *Oreochromis niloticus*, collected from Al-Khadoud spring channel at station III during the investigation period

Seasons	Liver	Muscles
Autumn	534.57±5.80 ^a	22.26±1.62 ^a
Winter	1050.27±7.70 ^b	20.52±1.21 ^a
Spring	882.70±5.70 ^c	10.72±1.45 ^a
Summer	917.93±5.93 ^d	33.14±0.15 ^a
Total average	846.37	21.66

Means ± SE (n = 10); Values within columns with no common superscripts are significantly different (p<0.05)

Bioaccumulation of metal within an organism results from interactions between physiological factors (growth, weight loss, absorption and accumulation), chemical factors (metal concentration, speciation and bioavailability) and environmental factors (temperature and food concentration)^[14].

The seasonally mean concentrations of the heavy metals (Cd²⁺, Cu²⁺, Fe²⁺, Mn²⁺, Pb²⁺ and Zn²⁺) in Al-Khadoud Spring water compared with WHO^[15] and USEPA^[16] allowable limits are presented in Table 1. The data shows that there is no difference between Cd²⁺ and Zn²⁺ concentrations on all stations. The seasonal concentrations of Cu²⁺ in water ranged between 0.005 at station I to 0.022 mg L⁻¹ at station III. However, Fe²⁺ concentration on station III was found to be much higher (2.210 mg L⁻¹) than other two stations. On the other hand, Mn²⁺ and Pb²⁺ values ranged from Non Detectable (ND) level at station I and II (0.017 and 0.021 mg L⁻¹, respectively) to 0.021 mg L⁻¹ at station III. The most pronounced feature is the highest concentration of Fe²⁺ in Al-Khadoud (2.210 mg L⁻¹) in comparison to other metals. Analysis Of Variance (ANOVA) of metals levels, showed no significant differences (p>0.05) among the stations, except for Cu²⁺ and Fe²⁺. The concentrations of metals in water were found in the following order:

$$Fe^{2+} > Zn^{2+} > Cu^{2+} > Pb^{2+} > Mn^{2+} > Cd^{2+}$$

The levels of heavy metals recorded in water in this study were generally low, when compared to WHO^[15] and USEPA^[16] recommended levels in water, except iron which was found to be higher than the recommended levels. The high level of Cu²⁺, Fe²⁺, Mn²⁺ and Pb²⁺ at station III could be attributed to discharge of either treated sewage water or re-use drainage water on the Al-Khadoud irrigation canal^[7].

In fish collected from the polluted station III, metal levels were significantly higher than the levels in water, indicating bioaccumulation. In the present study, the highest levels of metals were generally reported in fish liver than muscles (Table 3-5). Hepatic metal levels were ranked as follow: Zn²⁺>Cu²⁺>Pb²⁺>Cd²⁺. The fish liver plays a primary role in the metabolism and

excretion of xenobiotic compounds with morphological alterations occurring in some toxic conditions^[17]. Metals can either increase or decrease hepatic enzyme activities and can lead to histopathological hepatic changes, depending on the metal type and concentration, fish species, length of exposure and other factors^[18]. Regarding the fish muscle, Zn²⁺ was a little bit higher whereas Cu²⁺, Pb²⁺ and Cd²⁺ were characteristically low in the same site and Cd²⁺ was significantly the least. These findings suggest that heavy metals (like Pb and Cd), which have high affinity for thiol groups, turn proteins and peptides susceptible to structural modifications in sub-cellular compartments and tissues as in skeletal muscle. Some authors have already observed that cadmium alters calcium homeostasis^[19]. Inhibitory effects of cadmium, lead and mercury on Ca²⁺-ATPase activity from sarcoplasmic reticulum in rabbits were described^[20]. Morphological alterations in cultured skeletal muscle cell line as reflection of cadmium contamination altering the cell adhesion and the cellular antioxidant defense mechanisms were also described^[21].

Although the crucial role of zinc and copper in several enzymatic processes, they are tentatively classified as highly toxic metals by Hellowell^[22] and are bioaccumulated in aquatic organisms. Carino and Cruz^[23] suggested that higher concentrations of zinc impair reproductive success and survival potential of *Tilapia nilotica* in zinc-contaminated ecosystems. This heavy metal can exert adverse toxicological effects, when present in high concentrations in water^[24]. In fact, it is potentially toxic when the internal available concentration exceeds the capacity of physiological detoxification processes. On the other hand, organs of aquatic animals may accumulate copper when exposed to toxic concentrations^[25], which can lead to redox reactions generating free radicals and, therefore, may cause biochemical and morphological alterations^[26,27]. In addition, Pb residues could result in haematological, gastrointestinal and neurological dysfunction in animals. Severe or prolonged exposure to Pb may also cause chronic nephropathy, hypertension and reproductive impairment. Pb inhibits enzymes, alters cellular calcium metabolism and slows nerve conduction^[28]. In parallel, cadmium is a widespread environmental pollutant that is highly toxic and is considered to have no biological function^[29]. It causes severe membrane integrity damage with a consequent loss of membrane-bound enzyme activity which can result in cell death^[30]. This and other nonessential metals have been reported to cause anemia both in mammals^[31] and in fish^[32].

CONCLUSION

The present results showed that, the fishes, based on the higher levels of metal bioaccumulation, could be unsafe for human consumption. The finding is worrisome in view of the health implications for the population that depend on the spring for their water and fish requirements. Consequently, very close monitoring of heavy metal loads in Al-Khadoud spring is recommended in view of the possible risks to health of consumers.

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