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Concentrations of Copper and Zinc in Benthic Invertebrates Collected from the Tigris River at Baghdad City

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Abstract: The present study aims to assess the concentrations of Copper and Zinc in collected benthic invertebrates from Tigris River. Four stations were chosen on Tigris River along Baghdad City, the samples were collected every 60 days from November 2010 to September 2011. The benthic invertebrates which collected were: Bivalves: *Corbiculla fluminalis*, Gastropods:*Melanopsis nodosa*, *Theodoxusis jordani* and *Physa gyrina,* Oligocheates: aquatic worm, and dragon flies nymphs. The annual concentration of Cu in *C. fluminalis* soft tissue was 96.45 µg/g, while in the shell was 10.35µg/g. The Zn concentration in soft tissue was 201.7 µg/g, while in the shell was17.8 µg/g. In *M. nodosa* soft tissue had Cu concentration of 64.0µg/g, while the shell had 6.1µg/g. On other hand, Zn concentration in soft tissue was 297.5 µg/g, and in the shell was 21.1 µg/g., While in the *T. jordani* the concentration of Cu was 24.2 µg/g and the Zn concentration was 62.35 µg/g. Whereas in *P. gyrina* it found that the concentration of Cu was 32.4 µg/g , while the concentration of Zn was 76.2 µg/g. Aquatic worm had the concentration of Cu 127.0 µg/g and for Zn was 277.2 µg/g. The concentration of Cu and Zn were undetectable in Dragon flies nymphs that collected in spring and autumn. The benthic invertebrates, oligochaetes had the highest concentration of Cu, while *M. nodosa* had the highest Zn among the collected benthos, while soft tissue of bivalve *C. fluminalis* had the highest concentration of Cu and Zn than gastropods except soft tissue of *M. nodosa*. Depend on the statistical analysis results the soft tissues of bivalves and gastropods had higher concentrations of Cu and Zn than the concentration in the shell. In general the Zn concentrations in all collected organisms were higher than Cu concentrations.

***Keywords**: benthic invertebrates, Heavy Metals, Copper and Zinc, Tigris River.*

Introduction

The bio-monitoring of pollutants using accumulator species is based on the capacity which has some plant and animal taxa to accumulate relatively large amounts of certain pollutants. The use of this type of monitoring is widespread in marine and freshwater. This technique makes it possible to measure trace element concentrations even when their amounts in the natural environment are lower than the detection limits. In addition, the pollutant concentrations in the organism are reflects the recent pollution level of the environment in which the organism lives, while the pollutant concentrations in the water only indicate the situation at the time of sampling (Ravera *et al*., 2003).

Many researchers in the world study accumulation and biomagnfication of many compounds include heavy metals in aquatic organisms. Mason and Sheu (2002) had reported that mollusks have a depuration mechanism to reduce heavy metal toxicity in their body; this mechanism might diminish the effectiveness of molluscs as biomonitoring organism, as the concentration of heavy metal in the mollusca may not accurately reflect the concentration in the environment.

Ravera *et al.* (2003) study trace element concentrations in freshwater mussels and macrophytes as related to those in their environment, they showed that bioaccumulators can not be used to evaluate the pollutant levels of the environment at the time of collection, hence no relationship between metal concentrations in the species and those in the water was found and the relationship with the sediments was very weak, bioaccumulators can be regarded as a useful tool in long-term studies to follow pollutant variations in the same environment or when substantial differences in pollutant concentrations in different environments were found. Also Osman and Kloas (2010) have shown that

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the heavy metal residues in the tissues of *Clarias gariepinus* exhibited different patterns of accumulation and distribution among the selected tissues and localities, and reported that the liver was the site of maximum accumulation for the elements followed by gills while muscle was the over all site of least metal accumulation. Michael (2008) study trace metals (Zn, Pb, Cd, Cu and Ni) in the shells and soft tissues *of Tympanotonus fuscatus* var. *radula* from the mangrove swamps of the Bukuma oil field, Niger Delta, and found that accumulate Zn was more in the media, it was also higher in the seasons, stations and months of the study than the other metals. Few workers used invertebrates as bioindicator to determine the levels of heavy metals in Iraqi aquatic systems , Mustafa (1985) investigated the possibility of using the fresh water *Corbicula fluminae* as bioindicator for heavy metals pollution, he studied the Cd, Cu, Ni, Mn, Pb, and Zn elements in Shatt Al-Arab River , and found that there was significant correlation between the concentration of particulate heavy metals and those extracted from tissue, this could be attributed to the fact that this mussel is a filter feeder. Salman (2006) studied some trace elements in *Corbicula fluminae* and *Unio tigridis* which collected from Euphrates River, and found that there were high concentrations of heavy metals in their tissues. The main goal of our study was to investigate Copper and Zinc concentration in the collected benthic invertebrates from Tigris River that passed throw Baghdad city.

Materials and Methods

Four stations from Tigris River were chosen to collect water samples (Figure 1) from north to south of Baghdad City, the locations of these stations where: Station one (S1): located at the Al - Tajiyarea near Al-Muthanna Bridge, which is an agricultural area consists of groves of orange and other citrus tree.. Station two (S2): located at Al- Kharkh area under 17th July Bridge. Station three (S3): Located at Al-Jadriyah area near Al -Jadriyah Bridge. Station four (S4): located at Al- Rasheed area which is near AL-Zafarania City southern Baghdad City, which characterized, groves and homes for farmers beside the river. The vertical distance between Station 1 and Station 2 was 10.5 Km, while between Station 2 and Station 3 was 8.6 Km, and between Station 3 and Station 4 was 7.5 Km. Sampling was carried out bimonthly from November 2010 to October 2011. Extraction of heavy metals from invertebrates soft tissue of clams, snails, oligochaet, and aquatic insects larvae were prepared according to APHA (1992) whereas the shells of clams and snails were prepared according to Gonzalez *et al*. (1999) and measurement by using Atomic absorption spectrophotometer then final concentration measuring as the following equation:

$E_{con} = \frac{A \times B}{D}$ E_{con}: concentration of metal in sample (μg/g). A: Concentration of metal in calibration curve (mg/L). B: final volume of sample (ml). D: Dry weight of sample (g).

Figure 1. Map of Iraq and Baghdad shown the station on the Tigris River (Source: Ministry of Water Resource.2007. *Map scale 1/100000*)

Result and Discussion

In this study the benthic invertebrates which had been collected from Tigris River bank were: Mullosca [bivalves which include one species *Corbicula fluminalis* (MÜLLER); gastropods includes three species: *Melanopsis nodosa* **(**FERUSSAC**)**, *Theodoxusis jordani* **(**SOWERBY**)** and *Physa gyrina*] ; Annelids: Oligochaetes: aquatic worm; and Insects larvae include Odonata (dragon flies) nymphs. This study has detected the accumulation of Cu and Zn in the soft tissue and in the shell for two species *C. fluminalis and M. nodosa* which have shell, the concentration of the two metals revealed a seasonal variation. The concentration of Cu in *C. fluminalis* soft tissue was ranged 87.4µg/g in summer to 107.5 µg/g in autumn, and for the shell was from 7.1 µg/g in summer to 14.9 µg/g in autumn. While the Zn concentration of *C. fluminalis* soft tissue was from 120 µg/g in winter to 267 µg/g in autumn , while the shell was ranged from 8.0 µg/g in winter to 28.7 µg/g in summer (Figure 2 & 3). Statistical analysis showed that significant differences between seasons (p≤ 0.05) for Zn and Cu in shell and soft tissue (Table 1).

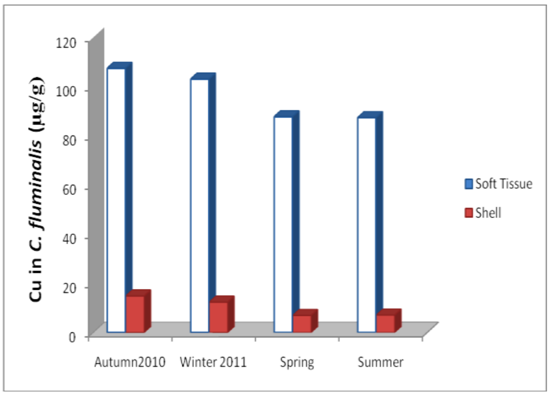

Figure 2. Seasonal variation of Cu in soft tissue and shell of *C. fluminalis* collected from Tigris River in Baghdad city during 2010 to 2011

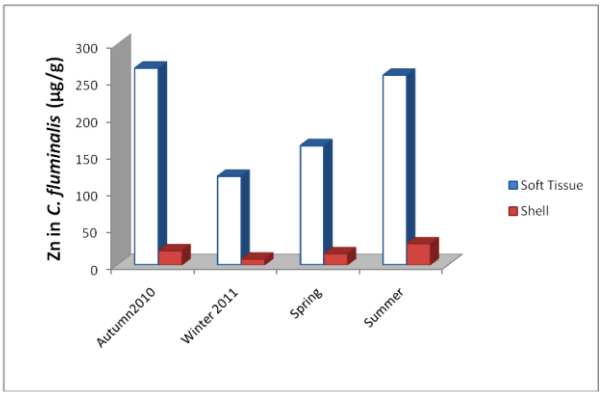

Figure 3. Seasonal variation of Zn in soft tissue and shell of *C. fluminalis* collected from Tigris River in Baghdad city during 2010 to 2011

Table 1. The concentrations of copper and zinc in *Corbicula fluminalis* soft tissue and shell collected from Tigris River in Baghdad city during 2010 to 2011.

Metal Con. Seasons	**Cu µg/g dry wet.**		**Zn µg/g dry wet.**	
	Soft Tissue	**Shell**	**Soft Tissue**	**Shell**
Autumn 2010	107.53±3.55	14.98±1.73	267.00±30.93	19.32±1.53
	93.40-123.10	8.20-23.10	190.30-414.00	14.20-27.30
	a	a	a	ab
Winter 2011	103.1± 3.72	12.42±1.89	120.42±9.68	8.05±0.678
	92.30-109.40	9.00-16.00	100.20-145.10	6.30-9.50
	a	a	b	b
Spring 2011	87.82±3.966	7.00±1.11	161.93±20.12	15.31±5.36
	70.30-104.00	3.00-12.60	101.2-277.30	5.60-50.00
	b	b	b	ab
Summer 2011	87.47±6.31	7.13±0.623	257.32±23.33	28.77±10.71
	73.30-104.00	5.70-8.55	214.00-307.00	16.20-60.80
	b	b	a	a

But Cu values for *M. nodosa* soft tissue was range from 44.0 µg/g in winter to 74.0 µg/g summer, and the shell varied from 6.0 µg/g in winter to 13.3 µg/g in summer. But Zn concentration for *M. nodosa* soft tissue was ranged from 260.0 µg/g in autumn to µg/g to 365.9 µg/g in spring ,whereas the Zn concentration in shell was lied between 12.1 µg/g in winter and 27.6 µg/g in autumn (Figure 4; 5). Statistical analysis showed those significant differences between seasons (P≤ 0.05) for Cu in shell and soft tissue, but no significant differences between seasons for Zn (Table 2).

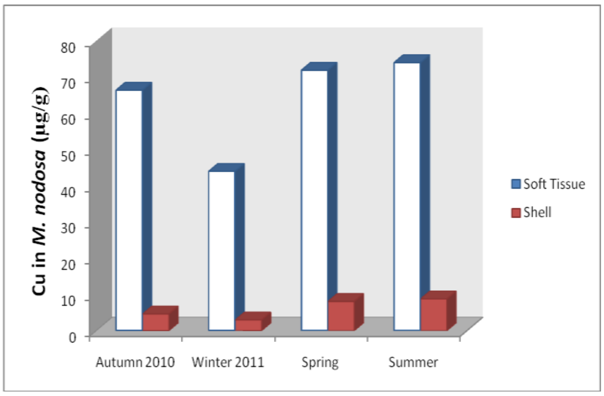

Figure 4. Seasonal variation of Cu in soft tissue and shell of *M. nodosa* collected from Tigris River in Baghdad city during 2010 to 2011

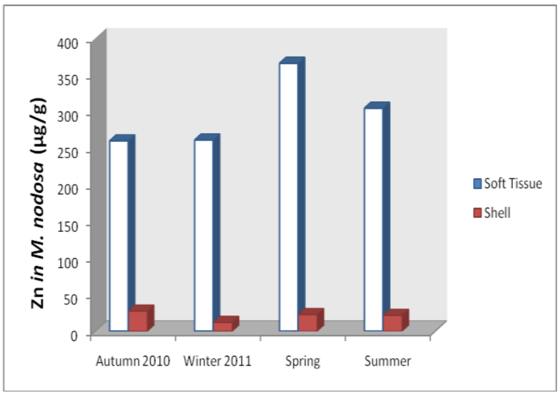

Figure 5. Seasonal variation of Zn in soft tissue and shell of *M. nodosa* collected from Tigris River in Baghdad city during 2010 to 2011

Table 2. The concentration of copper and zinc in *Melanopsis nodosa* soft tissue and shell collected from Tigris River in Baghdad city during 2010 to 2011.

Metal Con. Seasons	**Cu µg/g dry wet.**		**Zn µg/g dry wet.**	
	Soft Tissue	**Shell**	**Soft Tissue**	**Shell**
Autumn 2010	66.44±4.08 45.10-76.30 a	10.05±1.64 5.00-16.70 ab	260.01±54.75 66.10-503.00 a	27.60±3.45 18.00-44.90 a
Winter 2011	44.05±2.82 38.20-49.70 b	6.05± 1.17 3.10-8.80 b	261.27±53.58 166.30-387.50 a	12.10±2.13 9.10-18.30 a
Spring 2011	71.95±4.16 53.10-90.50 a	12.55±1.07 9.10-19.30 a	365.91±54.09 214.70-616.60 a	22.97±5.61 6.20-54.00 a
Summer 2011	74.00±3.39 68.50-80.20 a	13.31±2.21 8.15-18.20 a	304.70±65.49 185.40-411.20 a	21.80±6.19 10.70-32.10 a

The concentration of Cu in the *P. gyrina* was ranged from 14.8 µg/g in summer to 47.9 µg/g in winter. And in case of Zn was varied from 59.9 µg/g in summer to 96.2 µg/g in spring (Figure 6). Statistical analysis showed that significant differences between seasons ($P \leq 0.05$) for Cu and Zn, the lowest value were in summer (Table 3).

Table 3. The concentration of copper and zinc in *Physa gyrina* tissue collected from Tigris River in Baghdad city during 2010 to 2011

. Metals Con. Seasons	**Cu µg/g dry wet.**	**Zn µg/g dry wet.**
Autumn 2010	44.75 ±5.96 22.40-80.80 a	65.70 ±4.66 40.20-82.20 bc
Winter 2011	47.95 6.65 25.30-70.30 a	83.27 8.78 67.20-104.20 ab
Spring 2011	22.25 ±2.96 15.00-40.20 b	96.32±6.52 67.20-123.90 a
Summer 2011	14.82 ±4.37 7.60-27.40 b	59.95 ±4.98 50.40-70.70 c

The Cu values of *T. jordani* was ranged from 10.9 µg/g in spring to 31.9 µg/g in winter .While for Zn concentration ranged from 39.4 µg/g in winter to 83.17 µg/g in summer (Figure 7) . Statistical analysis showed that significant differences between seasons ($P \leq 0.05$) for Cu and the highest value were in winter. Also significant differences between seasons ($P \leq 0.05$) for Zn and the highst value were in summer (Table 4).

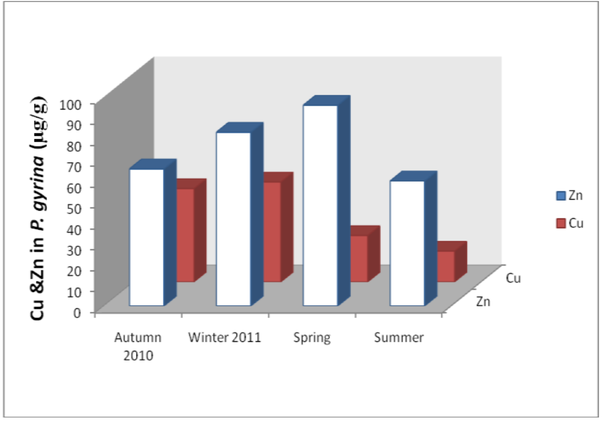

Figure 6. Seasonal variation of Cu and Zn in *P. gyrina* collected from Tigris River in Baghdad city during 2010 to 2011.

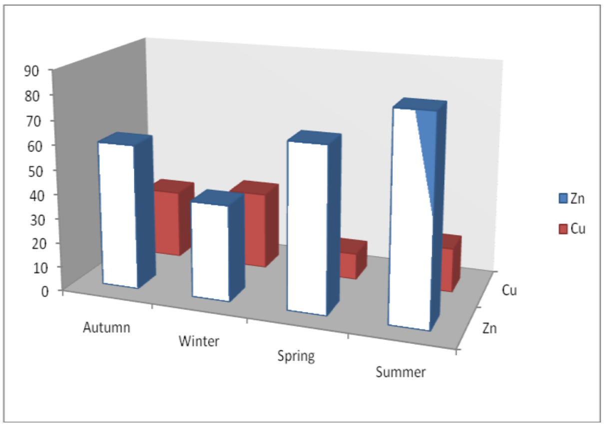

Figure 7. Seasonal variation of Cu and Zn in *T. jordani* collected from Tigris River in Baghdad city during 2010 to 2011.

Table 4. The concentration of copper and zinc in *Theodoxusis jordani* tissue collected from Tigris River in Baghdad city during 2010 to 2011.

Metals Con. Seasons	Cu µg/g dry wet.	Zn µg/g dry wet.
Autumn 2010	28.41 ±2.48	59.68 ±11.68
	18.30-40.10	26.70-107.40
	ab	ab
Winter 2011	31.90 11.19	39.0 ± 3.30
	15.10-66.00	24.60-38.80
	a	b
Spring 2011	10.97 ±2.24	67.34 ±18.11
	4.43-21.80	11.80-150.40
	c	ab
Summer 2011	18.10 ±4.45	83.17 ±15.31
	12.10-30.40	55.20-126.80
	bc	a

For oligochaetes tissue the Cu value was varied from 114.9 µg/g in Summer to 157.6 in Autumn .and for Zn value ranged from 166.4 µg/g in Winter to 355.1 µg/g in Summer µg/g (Figure 8). Statistical analysis showed that significant differences between seasons ($P \leq 0.05$) for Cu and the lowest value were in summer. Significant differences between seasons ($P \leq 0.05$) for Zn and the lowest value were in winter (Table 5).

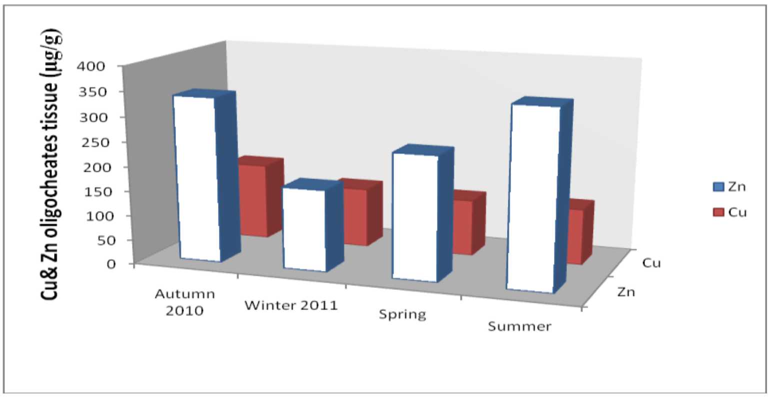

Figure 8. Seasonal variation of Cu and Zn in aquatic worm collected from Tigris River in Baghdad city during 2010 to 2011.

Table 5. The concentrations of copper and zinc in oligochaet′ tissues collected from Tigris River in Baghdad city during 2010 to 2011

Metals Con. Seasons	Cu µg/g dry wet.	Zn µg/g dry wet.
Autumn 2010	157.61 ±11.21	336.06 ±29.55
	91.00-190.70	223.20-443.00
	a	a
Winter 2011	122.02± 25.42	166.47± 20.44
	88.20-197.90	113.80-211.20
	ab	b
Spring 2011	115.56 ±6.34	250.43 ±26.62
	88.80-140.20	122.30-372.80
	b	ab
Summer 2011	114.97 ±4.04	355.10 ±56.46
	104.30-122.20	290.20-524.10
	b	a

The concentration of Cu and Zn in dragonflies nymph tissue were not detectable in those collected in spring and in autumn. The obtained results showed that Cu and Zn were accumulated in the body of the studied benthic invertebrates. In case of comparison of the concentrations of Cu and Zn in the filtered water and sediment, these metals are classified biochemically as essential elements in the bodies of living organisms and aquatic plants when present in trace amounts , but at high concentrations they become toxic (Kotickhoff, 1983).Also the aquatic organisms have high ability to detoxify the heavy metals by storage the metals into the binding with metal-binding proteins, metallothioneins (MT) and metallothionein-like protein, these proteins reported in mollusks, crustaceans, polychaets, and oligochaetes, metals which bind to these proteins are Cd, Hg, Zn, Cu, and Ag (Roesijadi, 1992). Cu and Zn are able to displace essential metals leading to threshold concentration of Cu and Zn in tissues,Metals are detoxified by binding to MT protein, while the precipitation of metals takes place into metal-rich-granules shown to contain Ag, Ca, Cd, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Pb, and Zn (Roesijadi, 1981; Brown, 1982).

From the current results, it seems clearly that the variations of Cu and Zn accumulated concentrations between the studied benthic invertebrates, and the most important causes of variability were: seasonal cycle, physical environment, and the biological state of the organisms. Species, size, age, and probably growth rate are the major biological factors influencing variability as suggested by previous study (Metcalfe-Smith *et al.* 1996). Bivalves have great accumulation capacity and low discrimination power (Bryan, 1979), his supports the present study finding where the concentrations of Zn and Cu in *C. fluminalis* were higher than in *T. jordani* and *P. gyrina* and higher in *M. nodosa* than *C. fluminalis.* Bivalve are useful pollutant indicators because of their wide geographical distribution, the presence of adults in all seasons, their sedentary way of life and can lived in both clean and polluted water body (Price and Pearce, 1997). Also the filtration activity and weak metabolism of bivalves allowed them to accumulate pollutants more than 100 to 1000 times than the surrounding environment (Hartwig, 1995). Bivalves and snails concentrate heavy metals through water and/or food, which make them available for biomonitors (Phillips, 1976; Phillips and Rainbow, 1993).

Study results showed that the variation in concentration of Cu and Zn in the species which had shell, particulary the shells which have a very slow turn-over time if compared to the soft tissues. Chemically calcium is very similar to zinc and copper and these metals all found in water in the form ions. The shell of mollusca consists carbonate due to absorbing $CaCO_3$ from the water (Lev *et al.*, 2007). The Cu and Zn can absorption through ingestion competes with iron, magnesium, and calcium (Kelly, 1988; USEPA, 2004). The metal composition in the tissue is a reflection of the recent situation while the shell reflects the integrated situation over a time period corresponding to the age of the bivalve (Mutvei & Westermark, 2001). Moreover to the high levels of Zn in the soft tissues than in the shells could be due to the role of Zn as an activator of many enzymes in the organs of some marine and fresh water organisms (Ireland & Kuwabara, 1985). The shell was used to show the relationship between the concentration of the metal in the soft tissue and in the shell, because it uptake of elements that take place from water and food, thus a fraction of these elements accumulated in soft tissue and part of the metabolized elements is transferred from the mantle to the shell (Ravera *et al.*, 2003). After metals being metabolized they were selectively concentrated in the soft tissue or in the shell (Ravera, 2001). The correlation between soft tissue and shell was (r=0.445) to Zn for *M. nodosa* and (r=0.458) for *C. fluminalis*, whereas the correlation to Cu was (r=0.447) for *M. nodosa* and (r=0.564) for *C. fluminalis* (Table 6). This observation is similar to that made by Dambo and Ekweozor (2000) in Oyster *Crassostrea gasar*.

Table 6. The correlation between copper and Zinc in the soft tissue and in the shell of *C. fluminalis* and *M. nodosa*.

	Shell (Cu)	**Shell (Zn)**
***Corbicula fluminalis* (soft tissue)**	0.564	0.447
***Melanopsis nodosa* (soft tissue)**	0.458	0.445

The current results indicate that mollusks have the high concentrations of Zn and Cu. This accumulation of this trace metals may be due to the low capacity of these mollusks for discriminating among metals which are similar in some characteristics such as ionic radius (Metcalfe-Smith, 1994; Jeffree *et al.*, 1993). Molluscs also possess a variety of effective detoxification mechanisms to reduce the toxicity of the metal uptaken (Byrne 2000; Byrne and Vesk 2000).

Benthic macroinvertebrates live or browse in the sediment and feed on trapped organic matter, worms and some bivalves feed on sediment and extract nutrients from its passage through the gut, detritus-feeding such as bivalve; gastropods; and crustaceans, ingest organic debris trapped in sediment, while other bivalves obtain food by filtration of large amount of water and collect the organic matter (Ridley, 1977). Mc Mahon and Bogan (2001) reviewed mentioned that *Corbicula fluminea* filter large volumes of water up to 2.5L water/h/clam. So they can pull water which contains phytoplankton, zooplankton, bacteria, organic debris, silt and clay. Snails feed on algae, bacteria, fungal films, and fine detritus (Beldi *et al.*, 2006). Oligocheata usually are collector which they are feeding on dead organic materials and bacteria in sediment (Gostafson, 1996).

The feeding mechanism plays an important role in metal uptake, bivalves ingest metals associated with organic and inorganic matter in the water column, whereas snails accumulate metals from periphyton and associated organic matter, while Oligochaetes ingest metals adsorbed to organic and inorganic sediment particles (Harding, 2005), from that ewe was explain that Oligocheates had the higher concentrations of Zn and Cu in their tissue among the studied benthic invertebrates. In addition to that the feeding habits, uptake of metals takes place through respiration across gills or skin surfaces (Elangoven *et al.*, 1997). Some benthic detritus not feed on sediment but on particles that they filter from overlying water (Walshe, 1947).

In spring and early summer an increase in metal concentration reflects an increased in requirement resulting from changes in metabolic activity (Bat *et al.*, 2000). Starvation and variation in weight of gonads during the reproductive cycle responsible for annual variations of the pollutant content, the seasonal differences in fat content influence their storage capacity, so the mussels are studied regards to the seasonal variation in pollutant content (Phillips, 1976). Cu and Zn are lipid solubility which explains the high rates of penetration across the cell membrane (Simkiss, 1984).

Benthic organisms are exposed to both particulate and dissolved forms of Zn in interstitial and overlying waters, as well as to sediment-bound Zn through surface contact and sediment ingestion. Dissolved forms of Zn are believed to be the most readily bioavailable (Campbell and Tessier 1996).

Copper and zinc, which are essential micronutrients, do not accumulate in decapods crustaceans, but are regulated up to certain threshold levels. Uptake at the cell membrane level is governed by specific carrier-mediated transport, transport through protein channels, passive diffusion of lipid soluble metals and endocytosis (Phillips & Rainbow, 1993). Zinc is used as an active centre for metaloenzymes and activators of other enzyme systems (carbonic anhydrate), while copper is an integral part of the respiratory pigment haemocyanin, accounting for the high copper levels observed at the hepatopancreas. Due to the fact that these metals are essential, they are also subject to strong regulation, being detoxified by metallothioneins (Canli & Stagg, 1997), eliminated by excretion through faeces or urine, and via haemolymph through excretory organs or gills (Arumugtan & Ravindranath, 1987).

Temperature affects the quantities of metal uptake by organisms because the increase in temperature may affect both influx and efflux rates of metals, while bioaccumulation may increase or not (Luoma, 1983). In this study the accumulation of Cu and Zn was various between seasons, it may be because the studied benthic invertebrates, depend of the collected benthic invertebrates from river shore at this season and this organisms may be not similar the previous organisms in size, age, and growth rate.

If the dissolved phase is the primary exposure vector, the metal concentrations in the molluscs should be correlated with the free metal ion concentration in the ambient water or its surrogate (Campbell, 1995). The review by Campbell and Tessier (1996) of bioassays and field surveys of indigenous benthic organisms supports the idea that benthic organisms respond to the free metal ion concentration in the ambient water in or near the surficial sediments, for field studies, the geochemical gradient (metalbio availability) has been defined in terms of the free-metal ion (as estimated from sediment-water equilibrium) or the ratio of sobbed metal to sorbent (related to the free metal ion concentration). The free metal ion concentration is a function of both the total aqueous metal present and the quantity and nature of Ligands present in the water. Consequently, it is not surprising that the free metal ion concentration can vary widely among systems as does the biological response it causes (Campbell & Tessier, 1996).

In the bioindicators it was impossible to establish any relationship between trace element concentrations and the present level of water contamination. Conversely, there was a weak similarity between element sequences in the organisms and in the sediments. However, this evidence of a possible, even though weak, relationship between bioaccumulators and sediments does not prove that the chemical composition of the organisms is more strongly influenced by sediments than by water chemistry. Despite living in contact with sediments, receive their main element supply from the water and suspended particles used as food. The process leading to element accumulation in sediments and organisms, such as Mollusk and Oligocheates, takes a relatively long time. Therefore the element concentration in the bioaccumulators can not reflect the water level of contamination at the time of sampling (Metcalfe-Smith, 1994).

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