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Investigation of Accumulation of Heavy Metals Cadmium and Mercury in Najaf Sea Through the Benthic Invertebrates for Bivalves and Gastropods

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Abstract: This study was conducted to assess the concentrations of cadmium and mercury in Najaf Sea at Najaf city through the accumulation of C and Hg in the soft tissue and in the shell for The benthic invertebrates which collected were: Bivalves: *Corbiculla* and *Unio*, Gastropods: *Melanopsis* and *Bellamya*. The samples were collected bimonthly from December 2016-October 2017 from four different select sites from Najaf Sea in Najaf city. The annual concentration of Cd in *Corbiculla* soft tissue was 0.094 μg/g while in the shell was 0.055 μg/g. While Hg concentration in soft tissue was 0.0341 μg/g while in the shell was 0.0069 μg/g. In *Unio* soft tissue the Cd concentration was 0.142 μg/g while in shell was 0.053 μg/g. Hg concentration in soft tissue was 0.057μg/g while in the shell was 0.021 μg/g. *Melanopsis* soft tissue had Cd concentration of 0.052 μg/g while the shell was 0.038 μg/g for Hg concentration in soft tissue was 0.0454 μg/g and in the shell was 0.0031μg/g. *Bellamya* was collected founding only in Spring and Autumn that the concentration of Cd in soft tissue was 0.087μg/g and in the shell was 0.051 μg/g while the concentration of Hg in the soft tissue was 0.038 μg/g and in the shell was 0.004 μg/g. The concentrations of Cd and Hg in all the collected benthos were found to be higher in Spring and Autumn than in Summer and Winter. *Unio* had the highest concentration of Hg among the collected benthos while *Corbiculla* had the highest Cd concentration. The soft tissues of bivalves and gastropods have higher concentrations of Cd and Hg than the concentration in the shell. In general the Cd concentrations in all collected organisms were higher than Hg concentrations.

Key words: Najaf Sea, accumulation, heavy metal, sources, benthic invertebrates, shell

INTRODUCTION

The Najaf Sea is considered to be the most prominent feature in the province of Najaf and the Middle Euphrates Region of Iraq. It is a manifestation of low terraces extending along the Euphrates River, separated not only by 15 and 16 km to the East while in the center 10 km (Jaafar, 2002). Crustal rock and the minerals it contains is the ultimate primary source of heavy metals in the environment. Background levels of cadmium and mercury in the Eearth's crust vary somewhat according to rock type and lie in the ranges of: Cd = 0.01-0.03 , Hg = 0.01-0.09 ppm (Brathwaite and Rabone, 1985).

Heavy toxic metal is any relatively dense or synaptic metal known for its potential toxicity, especially, in environmental contexts (Srivastava and Goyal, 2010). This term has special application on cadmium, mercury, lead and arsenic (Brathwaite and Rabone, 1985) all of which appear in the World Health Organization's WHO list of 10 chemicals of major general interest. Pollution and its effects are considered as one of man's greatest crimes against himself. Pollutants may cause primary damage, with direct identifiable impact on the environment or secondary damage in the form of minor perturbations in the delicate balance of the biological food web that are detectable only over long time periods (Sharma, 2012). Pollution of aquatic ecosystem by heavy metals is one of the most toxic substances that can bioaccumulation. Metals deposited into the aquatic environment may accumulate in the food chain and cause ecological damage, posing a threat to the human health duo of biological inflation over time (Benson *et al*., 2007).

The effect of cadmium and mercury on aquatic organisms depends on a variety of potential chemical forms of cadmium which may have different toxicities and different bioconcentration factors (USEPA., 2000; Al-Saadi, 2006). Benthic macroinvertebrates are common populations in lakes and streams where they are important in transmitting energy through food webs. The term "benthic" means bottom life, so that, these organisms usually inhabit the bottom substrate of at least part of their life cycle (Rosenberg *et al*., 1997).

In the aquatic environment an accumulation of abiotic and biotic chemicals may occur. The bioaccumulation of

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chemicals occurs within two or more physical components in the aquatic environment. Bioaccumulation involves the accumulation of chemicals within living organisms (Heng *et al*., 2004). Bioaccumulation becomes an environmental problem when chemicals accumulations are toxic which lead to an elevated amount in the organism's body. Toxicity may occur along the food chain when contaminants are consumed bioaccumulation of chemicals is used in the assessment of environmental hazard. Bioaccumulation has been found many aquatic biota as macrophyte, fish, invertebrates, benthic organisms, phytoplankton and zooplankton (Heng *et al*., 2004). The benthic aggregates contain types of different pollutant sensitivities and have been widely used to assess the environmental impacts of mineral contamination in the lake and streams (Maret *et al*., 2003). Metal contamination can reduce benthic macroinvertebrate species richness as well as density, growth and production (Maret *et al*., 2003; Gray and Delaney, 2008). Heavy metals can be accumulated in the gut and tissues of individuals (Sola and Prat, 2006).

In this study the benthic invertebrates which had been collected from Najaf Sea shore were: Mollusca (Bivalves which include the two genera: *Corbicula* and *Unio* and Gastropods includes: *Melanopsis* and *Bellamya*). This study was focused on the accumulation of Cd and Hg in the soft tissue and in the shell for those who have shell, the concentration of the two metals revealed a seasonal variation. The concentrations of Cd and Hg in all the collected benthos were found to be higher in Spring and Autumn than in Summer and Winter. *Unio* had the highest concentration of Hg among the collected benthos while *Corbiculla* had the highest Cd concentration. The soft tissues of Bivalves and Gastropods have higher concentrations of Cd and Hg than the concentration in the shell. In general the Cd concentrations in all collected organisms were higher than Hg concentrations.

MATERIALS AND METHODS

Description of study area: Sampling sites were located in the Najaf Sea of Najaf city Fig. 1a. (It is located between latitudes 32.4-31.45 and longitudes 44.6-44.29) in Iraq Fig. 1b (Jaafar, 2002).

Sampling was done for the period from December 2016 until October 2017, it was bimonthly at four different stations which they are (Fig. 2).

Sample collection: Benthic macroinvertebrates were collected at 4 sampling sites using a kick-net (1 mr1 m, 500 mm mesh). The organisms of each sample were preserved in 70% alcohol. In the lab, we rinsed the samples through a sieve (500 mm mesh). The retained invertebrates were sorted and identified generally to genus level.

Extraction of heavy metals from invertebrates: Soft tissue of clams, snails were prepared according to APHA, 1992 method for digestion. They were socked in distiled water for 24 h to eliminate any contamination from sediments and other materials in their guts, soft tissue was dried at 80°C for 24 h. 0.3 g dry weight digested by adding a mixture of HNO_3-$HClO_4$ in ratio 5:1 and heated at the oven at 90°C for 2 h, then lift overnight at room temperature followed by adding 5 mL of deionized water and centrifuge for 20 min at 3000 r/min. Then the volume was completed to 25 mL.

Shell of clams and snails were dried at the oven at 100°C for 24 h then broken up with ceramic mortar. About 0.5 g of dry wt. was taken, HNO_3 and HCl in ratio 7:2 were added and after that the sample left for overnight at room temperature in the hood. On the next day it was heated at 130°C for 2 h, then cooled, after that 5 mL deionized water was added and centrifuge for 20 min at 3000 r/min. The solution then completed to 25 mL in volume (Gonzalez *et al*., 1999).

Measurements of heavy metals concentration: Cadmium concentration measured by using atomic absorption spectrophotometer, Japan, 2004. Mercury concentration was measured by using cold vapor flameless atomic absorption spectrophotometer, Japan, 2002 with pump. Standard solutions were prepared for each metal by using same salts.

Concentration of metals was calculated from the calibration curve as described by Al-Taee (1999) as the concentration of heavy metals in soft tissue and shell:

$$Econ = \frac{A \times B \times df}{D}$$

Econ = 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)
df = Dilution factor

If used it was as the following:

$$df = \frac{\text{Volum of diluted sample solution in mL}}{\text{Volum of aliquot taken from solution in mL}}$$

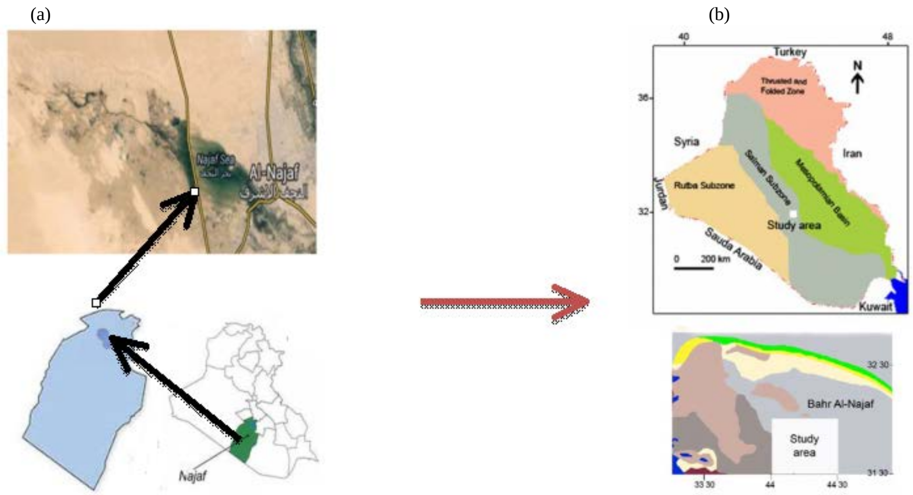

Fig. 1: a) A site map of the study area in Iraq and b) The sea of Najaf located from latitudes and longitudes

Fig. 2: The collection sites

RESULTS AND DISCUSSION

In this study the benthic invertebrates which had been collected from Najaf Sea shore were: Mullosca (Bivalves which include the two genera: *Corbicula* and *Unio* and Gastropods includes: *Melanopsis* and *Bellamya*). This study was focused on the accumulation of Cd and Hg in the soft tissue and in the shell for those who have shell, the concentration of the two metals revealed a seasonal variation. The concentration of Cd in *Corbicula* soft tissue was ranged 0.065-0.0812 µg/g and for the shell was 0.0359-0.0578 µg/g. *Unio* soft tissue was ranged between 0.092 and 0.154 µg/g while the shell content was ranged from 0.029-0.041 µg/g. *Melanopsis* soft tissue Cd was range from 0.017-0.075 µg/g and the shell was (0.0139-0.0361) µg/g. *Bellamya* benthic organisms founding only In April and October, the concentration of Cd in *Bellamya* soft tissue was 0.054 µg/g and in the shell was 0.025 µg/g. (Fig. 1-4 and Table 1-4).

The Hg concentration in the study findings showed that the *Corbicula* soft tissue contain 0.0095- 0.0389 µg/g and the shell was 0.0051-0.0075 µg/g while for *Unio* soft tissue the concentration was from 0.0232-0.0484 µg/g while in the shell was 0.0061-0.0107 µg/g for *Melanopsis* soft tissue Hg concentration was ranged from 0.0077-0.0156 µg/g in *Bellamya* soft tissue concentration between 0.0193 µg/g-0.0281 µg/g of mercury while the shell concentration from 0.0209- 0.0371 µg/g (Fig. 5-8 and Table 1-4).

Benthic macro invertebrates are common inhabitants of lakes and streams where they are important in moving energy through food webs. The term benthic means bottom-living, so, these organisms usually inhabit bottom substrate for at least part of their life cycle. The prefix macro indicates those organisms are retained by mesh size of 200-500 mm or can be seen by naked eye (Rosenberg *et al*., 2000). Heavy metals have non degradable nature, so have the risk of damage via. uptake and bioaccumulation by organisms which cannot effectively metabolized and excrete the absorbed metal (Oyewo, 1998; Otitoloju and Don Pedro, 2004).

Mc Mahon and Boyan (2001) reviewed mentioned that *Cobicula fluminea* filter large volumes of water up to 2.5 L water/h/clam. So, they can pull water which contains phytoplankton, zooplankton, bacteria, organic debris, silt and clay (Maclsaac, 1994).

Table 1: The concentrations of cadmium and mercury in *Corbicula* soft tissue and shell collected from Najaf Sea in Najaf city during 2016-2017

	Cd. µg/g dry wet.		Hg. µg/g dry wet.	
Metal con. seasons	Soft tissue	Shell	Soft tissue	Shell
Winter 2016-2017	0.0875±0.0011	0.0443±0.0020	0.0226±0.0010	0.0051±0.00016
Spring 2017	0.2132±0.0023	0.0965±0.0010	0.0389±0.0015	0.0072±0.00210
Summer 2017	0.0901±0.0150	0.0515±0.0018	0.0192±0.0082	0.0075±0.00110
Autumn 2017	0.0927±0.0180	0.0561±0.0031	0.0095±0.0011	0.0051±0.00030

Table 2: The concentration of cadmium and mercury in *Unio* soft tissue and shell collected from Najaf Sea in Najaf city during 2016-2017

	Cd. µg/g dry wet.		Hg. µg/g dry wet.	
Metal con. seasons	Soft tissue	Shell	Soft tissue	Shell
Winter 2016-2017	0.148±0.017	0.0379±0.0032	0.0232±0.0028	0.0061±0.00019
Spring 2017	0.1731±0.051	0.0493±0.005	0.0354±0.0175	0.0076±0.0021
Summer 2017	0.111±0.011	0.0681±0.0081	0.0484±0.0058	0.0095±0.0026
Autumn 2017	0.0132±0.013	0.0262±0.007	0.0197±0.0058	0.0107±0.0001

Table 3: The concentration of cadmium and mercury in *Melanopsis* soft tissue and shell collected from Najaf Sea in Najaf city during 2016-2017

	Cd. µg/g dry wet.		Hg. µg/g dry wet.	
Metal con. seasons	Soft tissue	Shell	Soft tissue	Shell
Winter 2016-2017	0.0271±0.0031	0.0142±0.0011	0.0077±0.0085	0.0018±0.0005
Spring 2017	0.0483±0.0018	0.0373±0.001	0.0156±0.0037	0.0062±0.0012
Summer 2017	0.0774±0.014	0.0155±0.0012	0.0094±0.003	0.0037±0.0006
Autumn 2017	0.0623±0.0044	0.0274±0.0017	0.0082±0.0019	0.0025±0.001

Table 4: The concentration of cadmium and mercury in *Bellamya* soft tissue and shell collected from Najaf Sea in Najaf city during 2016-2017

	Cd. µg/g dry wet.		Hg. µg/g dry wet.	
Metal con. seasons	Soft tissue	Shell	Soft tissue	Shell
Winter 2016-2017	N.F.	N.F.	N.F.	N.F.
Spring 2017	0.0321±0.0021	0.0132±0.009	0.0193±0.0017	0.0209±0.0022
Summer 2017	N.F.	N.F.	N.F.	N.F.
Autumn 2017	0.0761±0.0024	0.0364±0.0027	0.0281±0.0013	0.0371±0.007

NF = Not Found

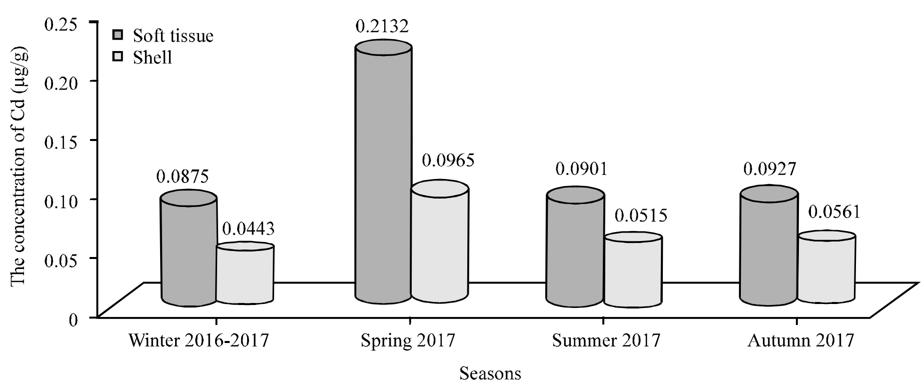

Fig. 3: Seasonal variation of cadmium in soft tissue and shell of *Corbicula* collected from Najaf Sea in Najaf city during 2016-2017

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 play 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). In addition to feeding habits, uptake of metals takes place through respiration across gills or skin surfaces (Elangovan *et al*., 1997). Some benthic detritus not feed on sediment but on particles that they filter from overlying

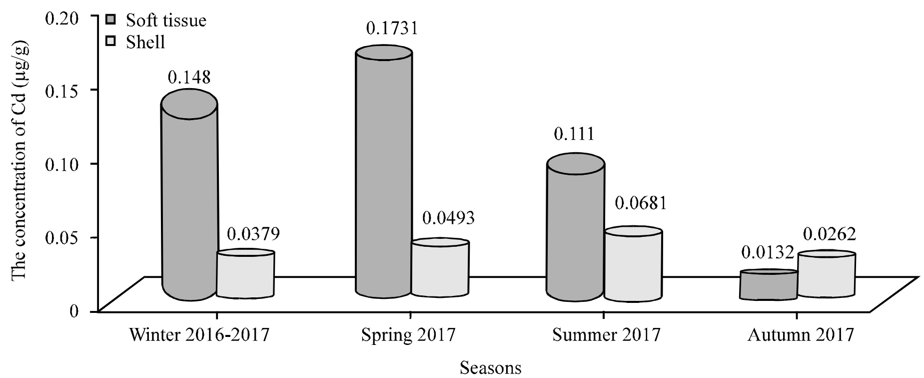

Fig. 4: Seasonal variation of cadmium in soft tissue and shell of *Union* colleted from Najaf Sea in Najaf city during 2016-2017

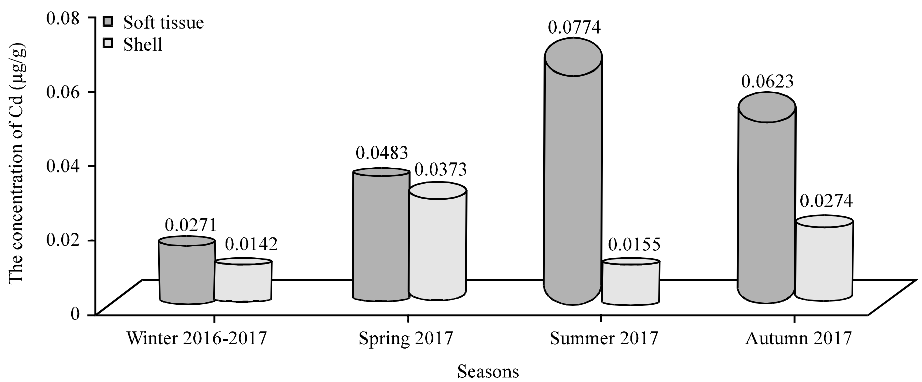

Fig. 5: Seasonal variation of cadmium in soft tissue and shell of *Melanopsis* is colleted from Najaf Sea in Najaf city during 2016-2017

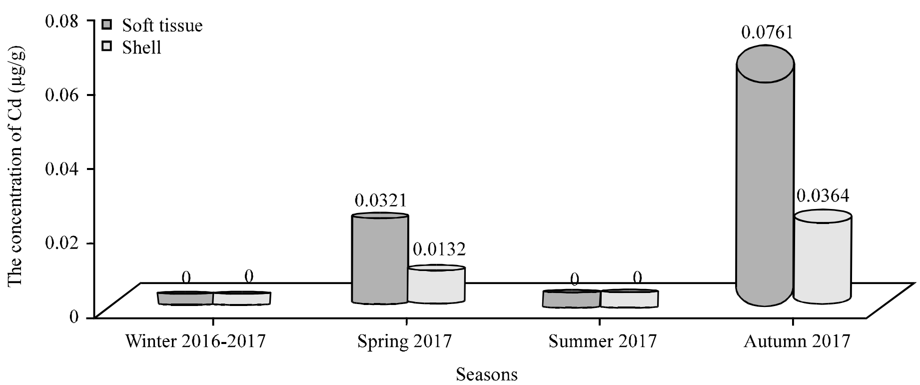

Fig. 6: Seasonal variation of cadmium in soft tissue and shell of *Bellamya* colleted from Najaf Sea in Najaf city during 2016-2017

water (Walshe, 1947). While Hare and Tessier (1996) stated that both water and sediment are the major contributors to metal accumulation. This may be explain the reason behind why the concentration of metals in two Bivalves *Corbicula* and *Unio* more than snail. Bivalves are known to concentrate various contaminants such as heavy metals enough to be use as an environmental indicator (Snyder *et al*., 1997). Due to the filtration

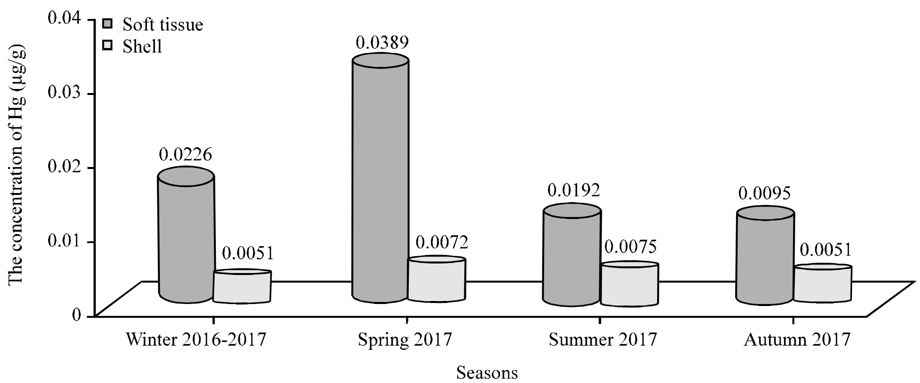

Fig. 7: Seasonal variation of cadmium in soft tissue and shell of *Carbicula* colleted from Najaf Sea in Najaf city during 2016-2017

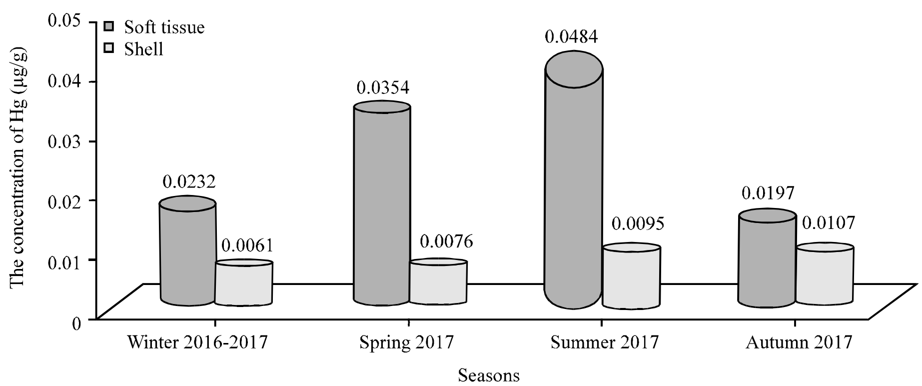

Fig. 8: Seasonal variation of cadmium in soft tissue and shell of *Unio* colleted from Najaf Sea in Najaf city during 2016-2017

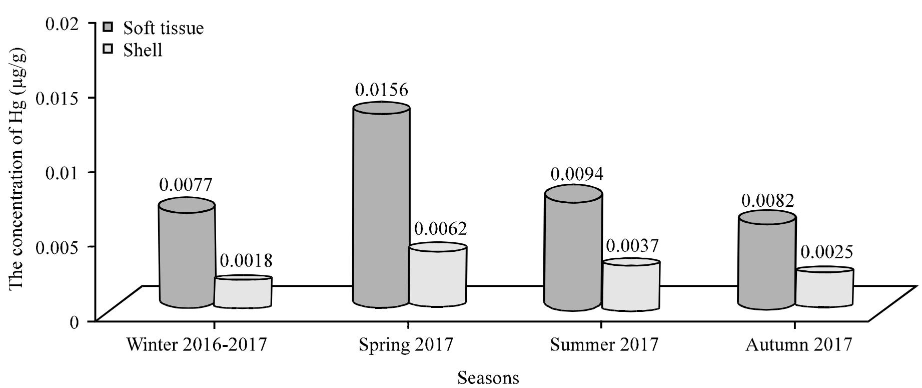

Fig. 9: Seasonal variation of cadmium in soft tissue and shell of *Melanopsis* is colleted from Najaf Sea in Najaf city during 2016-2017

activity, sedentary and weak metabolism of bivalves they can accumulate pollutants more than 100-1000 times than the surrounding environment (Hartwig, 1995). Bivalvates and snails concentrate heavy metals through water and/or food which make them available for biomonitors (Hartwig, 1995; Rainbow, 1995).

Detritus-feeding species uptake the metals in two major path ways: ingestion of metal-enriched sediment and suspended particles during feeding and uptake from solution (Luoma, 1989). Clams uptake the metal from particulate matter which they feed on (Nauen, 1983). Wetzel (2001) mentioned that the Cd uptake from prey is

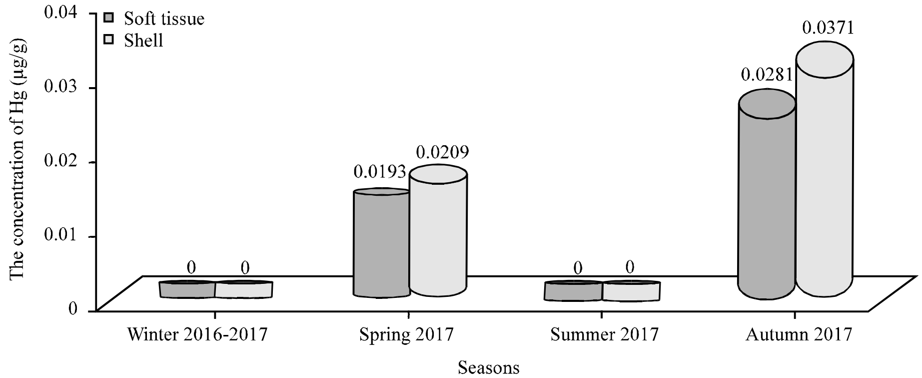

Fig. 10: Seasonal variation of cadmium in soft tissue and shell of *Bellamya* colleted from Najaf Sea in Najaf city during 2016-2017

as important the uptake from water. Other studies found that the sediment is the source of metals to the organisms (Abaychi and Mustafa, 1988; McCaulou *et al*., 1994). The sediment is the source of metals for Bivalves and Gastropods because they live in touch with the sediment (Rule and Alden, 1996; Cousins *et al*., 2002). While Lacastesantos (Lacastesantos-Fernandez, 2004) found that the concentration of metals in body tissue of mollusk related to the three environmental forms of metals in water, suspended matters and sediment and the concentration varied from species to another. Which is agrees with the finding of present study.

Factors such as pH, temperature, organic matter, clay and cation exchange capacity in sediment can influence bioaccumulation (USEPA., 2009). Salinity can affect the accumulation of Hg and Cd (Prosser and DeVillez, 1991). Statistical analysis of the data showed a direct correlation between pH and Cd concentration in *Unio* (r = 0.883). Also Cd found in *Melanopsis* soft tissue and shell was strongly correlated (r = 0.976, r = 0.91, respectively). While for *Corbicula* the Cd concentration had weaker correlation with pH (r = 0.532) while for Hg in *Corbicula* an inverse correlation with pH (r = -516) was found. The correlation between water temperature and Cd concentration in *Unio* revealed direct correlation (r = 0.992). Cd concentration in *Melanopsis* tissue and shell revealed a direct strong correlation with temperature (r = 0.859 and r = 0.962, respectively). While *Corbicula* had weaker metal correlation with temperature. 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 Cd and Hg was higher in Spring and Autumn than in Summer and Winter which may be related to the reproduction cycle where the animal activities increased in this period leading to increase uptake of metals. Body temperatures of invertebrates are the same as the water temperature, the metabolic rate will be higher in warmer water, so, more Hg will accumulate in Summer than Winter (Wright and Welbourn, 2002). Correlation with salinity was found to be inversely correlated for all organisms. *Melanopsis* had strongest inverse correlation between tissue and shell for Cd concentration and salinity (r = -0.854 and r = -0.981, respectively). *Corbicula* had weaker inverse correlation between Cd in tissue and shell with salinity (r = -0.774 and r = -0.736), respectively. The concentration of Hg in *Corbicula* was the lowest in relation to pH and temperature.

Mercury accumulated was varied with size and with age (Neumann and Ward, 1999). Parkman and Meili found that smaller benthos tend to accumulate less Hg concentrations. This agrees with this study findings which revealed that the concentration of Hg in *Unio* was higher than in *Corbicula* and *Melanopsis*, *Unio* is larger in size than the others. The mercury bioconcentration and bioavailablity are depending on the degree of methylation by bacteria and enhanced by the decrease in water hardness, alkality, pH, calcium and specific conductance (Wong *et al*., 1997; Lomniczi *et al*., 2004). Also affected by diet and growth rate (Verta, 1990). Cd also increased in soft tissue which is correlated with the age of animal (Noel-Lambot *et al*., 1978). In addition to that the body size, sex and season of sampling affecting accumulation of metals (Phillips, 1976; Srivastava and Goyal, 2010). Changes in tissue composition and reproduction cycle (Szefer *et al*., 2004). Luoma (1989) and Londis and Yu (1995) found an antagonism in uptake between Cd and Hg in some organisms. Smaller aquatic organisms accumulate metals more than larger ones, results from higher metabolic rates and a larger surface area to mass ratio, smaller prawn accumulate more Hg than larger ones

(McClurg, 1984). The present study finding showed that the Cd concentration in *Corbicula* tissue and shell was higher than *Unio* while Hg concentration was in *Unio* higher than in *Corbicula*. Accumulation strategies vary between pure regulation and net accumulation, depending on the biological species and the chemical element considered (Rainbow, 1988). Beside indirect uptake from food, accumulation depends on physiological characteristic of the organism and direct uptake from the water (Bouquegneau and Joiris, 1988). Statistical analysis of obtained data revealed a seasonal significant difference in all benthic organisms in this study ($p \leq 0.05$). This study findings in which *Melanopsis*, *Corbicula* and Unio increased in number in Spring and Summer while in Autumn *Corbicula* and *Unio* numbers decrease due to the disrobing sea in the study stations leading to damage the habitat for benthic invertebrates and may be cause death to *Unio* which found only the shell in the sea sediment. Bivalves have great accumulation capacity and low discrimination power (Bryan and Langston, 1992). 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).

The study results revealed that the concentration of Cd and Hg in soft tissue was higher than the shell in Bivalves and Gastropods. The shell and soft tissue typically differ in their chemical composition and the turnover time of the same element is much rapid in the tissues than the shell which is very slow, the metal composition in the tissue is a reflection of the recent situation while the shell reflect to the integrated situation over a time period corresponding to the age of the bivalve (Mutvei and Westermark, 2001).

The shell was used to show the relationship between the concentration of the metal in the soft tissue and in the shell because uptake of elements 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 Cd in soft tissue and shell in *Corbicula* was directly correlated ($r = 0.807$) while between Hg in soft tissue and shell was also directly correlated ($r = 0.739$). In *Unio* the correlation between Cd in soft tissue and in the shell was not found while the correlation between the soft tissue and the shell for Hg it was an inverse correlation ($r = -0.719$). In *Melanopsis* the correlation between Cd in the soft tissue and the shell was strong direct correlation ($r = 0.886$) while the correlation between Hg in the soft tissue and the shell was stronger direct correlation ($r = 0.965$). The correlation of Cd and Hg between the soft tissue and the shell in *Corbicula* and *Melanopsis* was direct correlation while for *Unio* was an inverse correlation, this may be due to the larger size and thicker shell for *Unio* than the others.

Lau *et al*. (1996) in Malaysia studied the concentrations of Cr, Pb and Cd in mollusks *Melanoides tuberculata* and the sea snail *Rapana venosa* and observed that metals concentrations in snail was lower than the other Mollusks. This agrees with this study findings which find that *Melanopsis* accumulate metals lower than *Corbicula* and *Unio*. Philippine government standards for Hg concentration in clams 0.5 μg/g, the Indian product legal limits for Hg is 0.5 ppm. Some species of clams decline as Cd concentration rose from 0.1-0.4 ppm (UNEP., 1999). USEPA (2002) water quality criteria standards for Cd, the acute criteria for freshwater life 2.0 μg/L and the chronic criteria 0.25 μg/L at hardness 100 mg/L.

CONCLUSION

The two Bivalves *Corbicula* and *Unio* and the Gastropods *Melnopsis* are available year around and have the ability to accumulate metals in detectable levels which make them a good bioindicators for Najaf Sea water quality. *Unio* had the highest concentration of Hg among all benthos while *Corbicula* had the highest concentrations for Cd. The benthic invertebrates which found in Najaf sea are tolerance to pollutants, indicating that the Sea is polluted.

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