Mercury biomagnification in Iraqi marshland (AL-Hawizeh (HZ) food chain using stable isotope analyses

Dr.Huda Farooq Zaki , Department of Biology,college of Science, AL-Mustansiriyah University, Baghdad 496. Iraq.

Dr. Reyam Naji Ajmi, Department of Biology,college of Science, AL-Mustansiriyah University, Baghdad 496. Iraq.

Abstract: A few studies have applied this technique in Iraqi marshland. The investigated Stable isotope relationships and mercury biomagnification food webs of five stations in AL- Hawizeh (HZ) marsh in (Algae , Macrophyte), invertebrates (Bivalve) fish and Birds)with mercury of particular importance in relation to food web, and impact of this contaminants on biodiversity .were analyzed .in this research was accurately described trophic structure by d15N, while d13C reflected the carbon source for each species. An increase of mercury levels was observed with trophic level, The carbon isotope compositions of marsh suggest that biota take nutrition from coupled food-web from primary producers, whereas the nitrogen isotope compositions indicate that birds occupy > five-level trophic positions . Results given us a positive correlation between Hg concentrations and d15N. Interpretation of the stable isotope data in combination with Hg concentrations in marshes. In the marsh is too much better understanding distribution of mercury in the food web and mercury contamination of the ecosystem. An evaluation was then made of how often the 15N and 13C values differed between types of biota in marshes. There was no statistically significant p value < 0.05 differences in 15N values between sites.

Keywords- Marshland ecosystems, Food web, Stable isotope, Mercury Bioaccumulation.

1. Introduction:

The marshlands are located in southeastern Iraq but also extend across the border into Iran. They once covered an area (20,000 Km2) between the three Iraqi Cities of Amarah in the north, Basra in the south, Naseriyah in the west , AL Hawizeh Marsh lies to the east of the Tigris River, straddling the Iran-Iraq border. [1].The marshlands constitute the largest wetland ecosystem in the Middle East. These wetlands are located at the confluence of Tigris and Euphrates rivers in southern Iraq. This area supported marsh Arab population of 500,000 as well as numerous endemic species of birds, mammals, amphibians, reptiles, fish and invertebrates. Pollution of aquatic ecosystems by elements is a global environmental problem [2] .contamination can have effects on aquatic organisms only after metal uptake and accumulation [3].In ecosystem. Primary producers (algae, macrophyte), invertebrates (Bivalve) fish and Birds were analyzed for total and organic mercury and for stable carbon and nitrogen isotopic. Carbon and nitrogen stable isotope measurements have been successfully used for determination the potential sources , as well as for assessing trophic levels in food webs, respectively [4,5] . The stable isotope composition of a consumer is the Weighting average of those of its food source in a predictive way [6,7] .(Hg) is an environmental toxicant of concern because of its pervasiveness and adverse effects on marshland [8]. In aquatic environments, upper trophic level organisms are exposed to MeHg almost exclusively via dietary uptake [9] MeHg concentrations that are potentially toxic to top-level consumers, [8, 10] monitoring mercury contamination in aquatic environments as marshes is necessary to properly assess risks to ecological [11] .Marshes biota and these may experience increased environmental MeHg exposure. In this study, we determined the stable carbon and nitrogen isotopic compositions, and Hg concentrations, in 150 specimens collected from throughout the Hawizeh (HZ) marsh. Aims our study to investigate the influence of trophic position and food-web structure on Hg concentrations in food chain Biota.

2. Material and Method

2.1: Site Description:

The study areas five stations are point along the major marshes AL-Hawizeh (HZ) in southeast Iraq. This marsh could cover about 3,000 km2. [12]. Hawizeh (HZ) (IBA 032 & 036), Admin Area: Missan and Basra , Central coordinates: N 31º 27' 00" E 47º 39' 00" , Area: 165,000 ha . Status: Unprotected , Altitude: Around 4 m ,Ecoregion: Tigris-Euphrates alluvial salt marsh, Directional information: This area is located approximately 40 km southeast of Amarah city and 60 km northwest of Basra.

Table 1: Shows the coordinates of the study areas of AL-Hawizeh marshes.

Sub-Site Code	Sub-Site Name	Nearest Town	Coordinates
HZ1	Umm Al-Ward Bushes	Kahla	N 31 34 5 E 47 30 4
HZ2	Majnoon	Al-Deer	N 31 5 41 E 47 34 38
HZ3	Umm An-Ni'aaj	Kahla	N 31 35 35 E 47 34 56
HZ4	Udheim	Musharah	N 31 41 13 E 47 44 56
HZ5	E'jayrda	Ezeir	N 31 19 55 E 47 37 51

2-2: Samples Collection

These samples (Algae, Macrophyte, Bivalves, fish and Birds) were collected at 5 major stations from AL- Hawizeh (HZ) marsh it was used to calculate the differences in 15N and 13C between consumer analyses and algae, macrophyte in producer. The study was conducted in that for after 10 years following restoration happened 2003 received continuous mercury discharges from two important rivers Tigris and Euphrates. Mercury contamination problems still occur as a result of internal loading from the sediments. Sampling was conducted in the most contaminated area of the marshes, in late spring/early summer 2013 when algal presence is common. Primary producers and consumers were randomly collected (three replicates consisting of composite samples) from different location were collected from the same station. Samples of birds were adult individuals (18 months average age), while the Fishes were adult individuals (Using the growth rings on the scales of fish or structures ringed otic (tiny bones in the inner ear and every pair of rings represents a year from the age of the fish. Because rings squamous affected sometimes by other factors so we used stones otic to estimate the age of the fish, which indicate structures ringed also years of age fish). Sampling of Bivalve focused on 3-year-old adults (about 3 cm shell width) freeze-dried and analyzed whole (bivalve shells removed) for mercury and stable isotope ratios. Muscle samples were obtained from the species was previously identified from stomach content analysis. They include Birds (*T. altirostris* and *A.griseldis*) , Fishes (*Liza abu, Silurus triostegus*) and Bivalve after removal shell (*Unio Tigridis, , viviparous bengalensis)* , and digest parts of Aqutic plants (*T.domingensis, C.demersum*) and Algae(*C. nivalis , C.reinhardtii*). All samples were stored at −20 ◦C until analysis [13] three samples of each organism were captured in low water conditions to got 150 total samples.

2.3. Stable isotope measurements:

Stable isotopes measurements of carbon and nitrogen were carried out in muscle samples Birds, Bivalve and fishes. After being dried at 60 ◦C (72 h), samples were into a homogeneous powder. Since all muscle samples from the present study presented low lipid content (C:N < 3.0), no lipid extractions were carried out [14]. Stable isotope Reference materials Standers (IAEA CH-6 and IAEA-N1) were also analyzed and the precision of replicate analyses was 0.5%.Stable isotope ratios are expressed in delta notation as part per thousand. Carbon and nitrogen ratios are expressed relative to the V-PDB (Vienna Peedee Belemnite) standard and to atmospheric nitrogen, respectively. Stable isotope analyses were performed on freeze-dried Aquatic plant and Algae samples using a stable isotope ratio mass spectrometer for RMS Delta V advantage Thermo. Carbon (d13C) and nitrogen (d15N) ratios were expressed as the relative difference

(0.5 %). The trophic magnification factor (TMF) of the marshes food web was calculated for both total and organic mercury, through [15,16,17] .Stable isotope analysis was used to assess the effect of trophic processes on the Hg content of biota [18,19,20] .

The recovery of internal reference material for the CF-IRMS method was 99.7% and 99.6% for nitrogen and carbon, respectively to muscles tissue Moreover, the mean sample precision determined from duplicate analyses was 96.5% (range = 91.3–100.0%) and 93.8% (range = 90.4–100.0%) for nitrogen and carbon, respectively to macrophyte and Algae .

2.4. Total mercury (THg) determination:

Aliquots of approximately 0.4 g of samples digested with 1mL of hydrogen peroxide and 5mL of sulfuric:nitric acid mixture (1:1). The solution was then heated to 60 ◦C for 2 h in a water bath, which was followed by the addition of 5mL of potassium permanganate 5% solution and heating to 60 ◦C for more 15 min. After overnight digestion, THg concentration was determined by Cold Vapor/AAS (FIMS-400, Perkin-Elmer) with sodium borohydride as reducing agent. Blanks were carried through the procedure in the same way as the sample. The standard reference material DORM-3 was analyzed in every run and our results were in good agreement with certified values (Mean recovery ±S D = 0.150 ±3.89%).

2.5: Statistical analysis

Mean carbon and nitrogen isotopic values were calculated or each species as sigma plot test was used in order to test for normality of the data. ANOVA and used for comparing nitrogen and carbon isotopic values among feeding types Simple linear regression analysis was used for investigating relationships between 15N and logarithmic concentrations of THg, as well as for determinating trophic magnification factors (TMF). TMF is calculated as the anti-log of the regression slope with base 10 and can be used for quantifying food web biomagnification [15,21].Therefore, this tool was used for calculating Hg biomagnification in different ecosystems.

3: Results and Discussion

3.1. Nutritional Relations

Stable isotope results (Figure 1; Table 2) appear in five marsh stations d13C (from _18.59 to 0.26%) values d15N (17,39-, 29%) and d13C average is - 15.9 ‰ (1.7% SD) The value d15N average 14.5% (1.1 ‰ SD) of 150 samples analyzed. From the results obtained differences in the structure, different sets of samples (birds, fish and animate) and (Macrophyte and algae) data from the same region itself there are similar spatial differences in some aspects of the food web structure, but not the same as to whether this difference to the nutritional differences or food differences . The isotopes of carbon and nitrogen due to various food relations in food webs but at different levels of degradation [22]. These values ranged between _23.40 to 0.18% and 0.26% _17.61 12.5 to 15.6 ‰ ‰ and nitrogen isotopes ranged from 3.4% to 10.3% of these species.

In addition, the birds showed the highest 15N fish and Bevalve (from 13.7% to 16.7% and 13.7% from 16.8% for those types of macrophyta to algae from 15.5% to 16.5% (P <0.0012). The fact that the object of the present marsh food web relatively low nitrogen isotope values probably reflects high. This point to refer to the state of deterioration is much higher in the marshes. Average high results indicate 15N fish as an important prey for birds (16.67) sampling, has been verified from the top 15 N in fish (13.63), 13.99 animate, Macrophyte (11.20), indicating that the species as a bird was on the upper part of the network food, the highest mean 15 N value of those web at each station.

species	*Type organism*	*Total(Hg,MeHg) SD ±Mean*	*d15Na SD ±Mean*	*d13Ca*	*Trophic Magnification factors (TMF)*	*p-Value*
T. altirostris	***Birds***	**0.147± 0.027**	**16.67± 0.33**	**23.40± 0.18**	**1.69**	**0.0994**
A.griseldis	***Birds***	**0.155 ± 0.031**	**17.39 ± 0.29**	**21.33± 0.22**	**1.54**	**0.0895**

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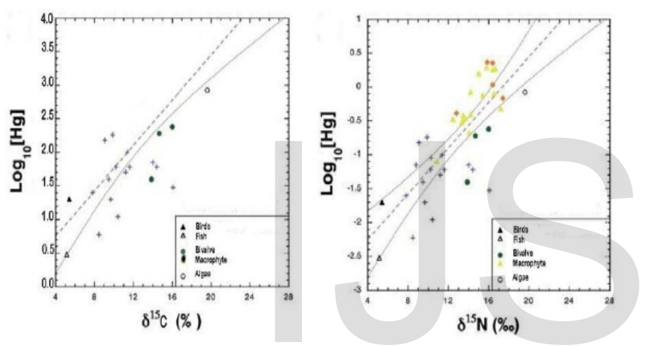
Unio Tigridis	***Bivalve***	**0.138± 0.015**	**14.67± 0.27**	**17.54 ± 0.47**	**1.71**	**0.0745**
viviparous bengalensis	***Bivalve***	**0.126± 0.019**	**13.99 ± 0.19**	**18.64 ± 0.39**	**1.64**	**0.0532**
Liza abu	***Fish***	**0.076± 0.017**	**13.63 ± 0.25**	**23.46 ± 0.22**	**1.50**	**0.0353**
Silurus triostegus	***Fish***	**0.096 ± 0.014**	**11.83± 0.17**	**22.16± 0.22**	**1.42**	**0.0635**
C.demersum	***Aquatic plants***	**0.129± 0.032**	**11.20 ± 0.23**	**18.59 ± 0.26**	**1.59**	**0.0746**
T.domingensis	***Aquatic plants***	**0.187± 0.036**	**11.05± 0.41**	**17.61± 0.26**	**1.17**	**0.0434**
C. nivalis	***Algae***	**0.152 ±0.022**	**-**	**-**	**1.24**	**0.0242**
C.reinhardtii	***Algae***	**0.147± 0.029**	**-**	**-**	**1.33**	**0.0340**

Table 2: show total mercury (Hg; THg ppm dry wt.), stable nitrogen (d15N) and carbon (d13C) isotope, Trophic Magnification factors (TMF), collected from the AL-Hiwazah marshes. (3 type of 5 region each one = 150 samples)



Total Mercury concentration Stable Carbon (d15C) Isotope in Biota acummulation factor   Total Mercury concentration Stable Nitrogen (d15N) Isotope in Biota acummulation factor

Fig :1: show total mercury (Hg; THg ppm dry wt.), stable nitrogen (d15N) and carbon (d13C) isotope, Trophic Magnification factors (TMF), collected from the AL-Hiwazah marshes.

3.3. Hg and THg–relationships with TMF

Mercury concentration in the different species increased with increasing trophic level in the marsh, with the exception of those types of types of algae. other less 15N average as well as the lowest concentration values THG, while fish and Bivalve with the highest concentrations of nutrients showed 15N THG average ($P < 0.0009$). And found a positive correlation between the concentrations of THG and 15N values for marsh $P < 0.005$). Trophic magnification factors (TMF) above (Table 3), refers to the accumulation of mercury in web, and revealed that significant exposure to mercury path [23.24], give us a species and birds most fish, and higher concentrations THG were found in marshes ($p < 0.05$). It becomes to look at it, in the marshes, and are exported mercury content received from organisms to be organic materials [25]. The findings revealed the carbon and nitrogen isotope relations in various food webs of the differences in the marshes biomagnification processes TMF among the five food webs indicate that the characteristics of each station is likely to affect the TMF mercury contained. In this study, it was revealed that there no statistically significant relationship between total mercury of biota due to the different specific types.

5. Conclusion:

The high results differed between the species: (1) the bird facing additional absorption of mercury by eating fish directly and type of Bivalve and algae macrophyta (2) the organisms that keeps the food is relatively high level in the food web. As were all mercury pollution evaluation mercury concentrations own varieties of birds, TMF-normalized total mercury and methylmercury. With the exception of algae, and is associated with the total mercury varieties which indicates that the organisms were identified appropriate indicators of environmental mercury pollution. Biota in marshes represent important targets for programs mercury monitoring, as well as stable isotopes represent this study include the first different types of food chain and signatures isotopes large analysis (D13C and d15N) with the surrounding Iraqi Marshlands area conditions despite the fact that these areas are closely linked with the life of the Tigris and Euphrates rivers is clear from the results that the main factor in influencing the difference is the spatial distribution and type of nutrition for the organism, Total mercury concentration in the foods web indicates that biomagnification of mercury and this shows that the efficiency of the transfer of pollutants is to increase the efficiency of biomass transfer (biomagnification), [3,9,21].General and biomagnification of mercury through several groups (algae, fish, Birds, Bivalves and plants). We have no description biomagnification of mercury does not reflect the ability to be amplified in certain subsets of the food web. This suggests. There are different transports mechanisms that occur in may be different levels of the food web. This probably refers to some physiological shift in the metabolism with the progress in the food chain, or a change in the size or trophic level ratio should be focused on the use of stable isotope technique to further explore the behavior.

5. Recommendation

There is a need to further study to determine the potential of the various sources of this element in the region, effects, and how it affects living organisms, including the development of the monitoring program: mammals more, birds , fish, plants, soil, water, sediment and more invertebrate samples should be collected and analyzed in order to obtain a more complete indication of the pollution risk element in the marshes. Additional samples taken priority should be given to this site should be chosen additional sites in the vicinity of the contaminated marshes happen. It is recommended that the various elements of the levels of stable isotope analyzes also (as lead, chromium ...) to be checked in the region, and also consider increasing the number of sites evaluating and selecting new sites based on the potential spatial patterns of pollution sources of pollution in the marshes.

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