Assessment of (comet assay) in some aquatic species (*Tilapia zilli, Aspiusvorax, Uniotigridis,Sesarmaboulengeri)*as molecular biomarkers of lotic aquatic system

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Abstract :

This study was designed to evaluate of some environmental molecular biomarkers such as comet assay in some two species of fish (*Tilapia zilli ,Aspiusvorax*),theClam(*Uniotigridis*)and the Crab (*Sesarmaboulengeri*) which were collected from three sites in Hilla river. We determine the three paramrters in comet assay, by comparative with control for these species. Depending onTail length(µm). as indication of DNA damage, it has the highest level in st.1 in the species *S. boulengeri, T. zilli,U. tigridis* at respectively, and the highest level of DNA damage in the st.2 in the species *S. boulengeri, U. tigridis A. vorax* at respectively, and it highest level in the st.3 in species *S. boulengeri* This study indicates that the molecular markers different in response in aquatic organism

Key word : comet assay , aquatic organisum , molecular biomarker, Hilla river

Introduction

The aim of molecular biomarker is to improve a sequances of sensitive molecular assay that will lead to the rapid tetection and make progress for the properties of the pollution to which the organism is reaction , Molecular biomarker such as DNA strand breakage accomplished by many researchers like (Black, etal.,1996).

Using molecular biomarker to assess ecosystem or organismal health is a popular concept there is abundant evidence of the effectiveness in specific case studies of single species (Feder*etal*., 1999).The molecular biomarkers is allow scientists to diagnosis a potential environmental problem to any phenotypic or toxicological expression in the organism,on this basis providing an early warning system (Obiakor,2012). Studying of (Salman *et al*., 2012) which is designed to detect the effect of heavy metals on the characteristics of the DNA in common fish Cyprenuscarpio by copper and cadmium, respectively, with acute exposure(96 h) and the results were seen that DNA damage fluctuated according to different exposure concentration therefore some lanes showed high fragmentation than another in both fin and superficial tissues

The study on the toxicological effects of aquatiuc contamination have been performed on avariety of bioindicators specially in fish and bivalve mollusks

Material & Methods

The study was included three sites on Al-Hilla river from north of city which is the first site (Sinjar region), this site is far about (7 Km) from the center of Hilla city , second sites (Al- farsi region) is far at south of the city, and third site (Hashemia region) which far about (35 Km) of the center of Hilla city , and the variability of the sites are according to specification in component and diversity of living organisms.(Figure 1) .

Comet assay

This assay has been done according to Comet Kite (Single cell gel Cat. No. 0905-050- K) used to identify DNA damage and we followed the protocol clarified by The steps of Comet assay was Followed as:

Tissue Preparation

Liver –nerve node

1-After the animal has been humanely killed ,then tissue is removed and kept on ice

2-Asmall portion (50)mg is serially washed in a large volume of ice cold mincing solution to remove excess of blood.

3-The piece of liver or tissue is placed in a 2.0 ml microcenterfuge tube containing 1.5 ml of ice cold mincing solution , the tissue is minced for 1-5Min.

4-Wait 5 minutes allowing depris to despit at the bottom , then for each slide transfer 2-5 µl of the cell suspension to a clean 1.5 ml tube and mix with LMPA agarose.

Mussel Hemolymph Cells

1-Add 40 µl of o.5 M di- sodium EDTA (Cat . No. 0905-050-04) to 1.5 ml microcentrifuge tube.

2 .Fill a syringe with 0.5 ml of a mussel physiology saline solution PH 7.40

3- Collect o.5 ml of hemolymph from the sinus of the valvar posterior abductor muscle , then transfer the whole content of the syringe to the normalized microtube.

4- Mix up to 7.5 µl of cell suspension with 75µl of LMPA agarose. All steps are performed at 18-24 C^{o} unless otherwise specified , in a low humidty environment . Work under dimmed or, yellow light to prevent damage from UV.

These tissues it possible preparation to comet assay DNA by followed as

ALKALINE ELECTROPHORASIS

1- Prepare lysis solution and cill at 4 Cº or on the ice for at least 20 minutes before use if it has been srored room temperature

2-Melt LMPA agarose in 95 Cº hot water for 15 minutes with the cap loosened ,mixing at interval . if not compelety molten , place it at 95Cº for 5 minute more . Equilibrate LMPA bottle in a 42 Cº water bath for 10 minutes.

3-A maximum volum of 7.5 µl (cells) with 75 µl molten LMPA agarose (at 42 Cº) and mix it onto the clear part of a comet slide that has been labeled by scratching with a point.

4-Warm comet slide over a heating plate at 42- 50 Cº before application this will prevent permit evenly spreading of the agarose and prevent air bubbles formation.

5-Take a 24× 50 mm cover slip between thumb and first finger ,match it to the upper horizontal edge of the slide close to the right side and make fall the cover slip over the slide

6-Place slide flat in a stainless steel tray and transfer at 4Cº into the refrigerator for 5 to 10 minutes , or leave at RT for 15-20 min.

7-Carefully ,remove the cover slip and place another 100-200 µl of LMPA

8-Remove cover slip and immerse the slide in prechilledlysis solution at 4Cº for 60 minutes, in the dark. Incubation may be conveniently performed into a vertical or horizontal jar for forhistochemistry .

9-Tap off excess buffer from the slide and immerse in freshly prepare Alkalin solution , PH > 13.

10-Leave comet slide in alksline solution for 5 minute at room temperature in the dark .

11-(Optional air dry samples . Drying bring all the cell in single plane to facilitate observation . Samples may be stored at room temperature.

12-Remove slide from alkaline solution , gently tap excess buffer from slide and wash by immersing in 1X TBE buffer for 5 minute ,2 time.

13-Transfer slide from 1 X TBE buffer to an horizontal elecrophorasis apparatus.

14-Power supply to 25 volt per cm , 38 Amper.

15-Remove excess solution turning the slide on a side and placed 300-500 ml of diluted FLUO plus dye on to the slide . Incubate 2 to 5 min. Tap excess dye and place a cover slip . if the slide was dried in ethanol.

16-View slide by fluorescence microscopy at 250- 1000 X magnification , depending on dimension of cell nuclei The data.

possible analysis according to comet score version by followed as:

Tail length= length of tail measured by ocular micrometer (µm)

DNA Tail Moment (%) = the product of distance and normalized

(intensity integrated over the tail length, Σ (Lx. %DNAx)

(Comet Assay Index = (width of head / length of tail)

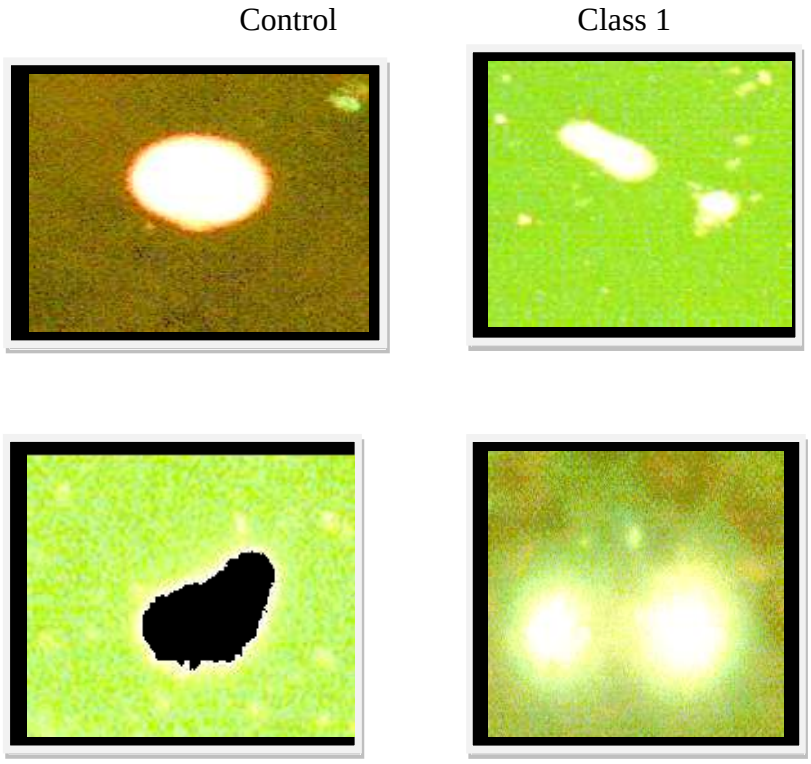
Classes of damage = according to comet assay index :1.2 – 2 (Low

damage), 2.1- 3 (Medium damage) , up to 3 (High damage).

Fig. (1): Satellite Image of study sites on Hilla River, middle of Iraq

Results:

In table (12) and according to the pictures that were taken by microscope flourcenes type leica company ,germany) and taken by it (50) pictures were depending on samples of four species under study (fishes: liver) and Clam (U.tigridis : hemolymph) and Crab freash water (S.boulengeri : nerve node) in March 2015 , This can be illustrated species and varieties of damage based on the comparison with control According to picture were taken can be classified into class 1,class 2, class 3 (Figure) and after the results of statistical analysis and calculation of the cells according to the measurements (comet length.tail length µm ,moment %) and that shown in the statistical analysis made clear case (high comet assay index).

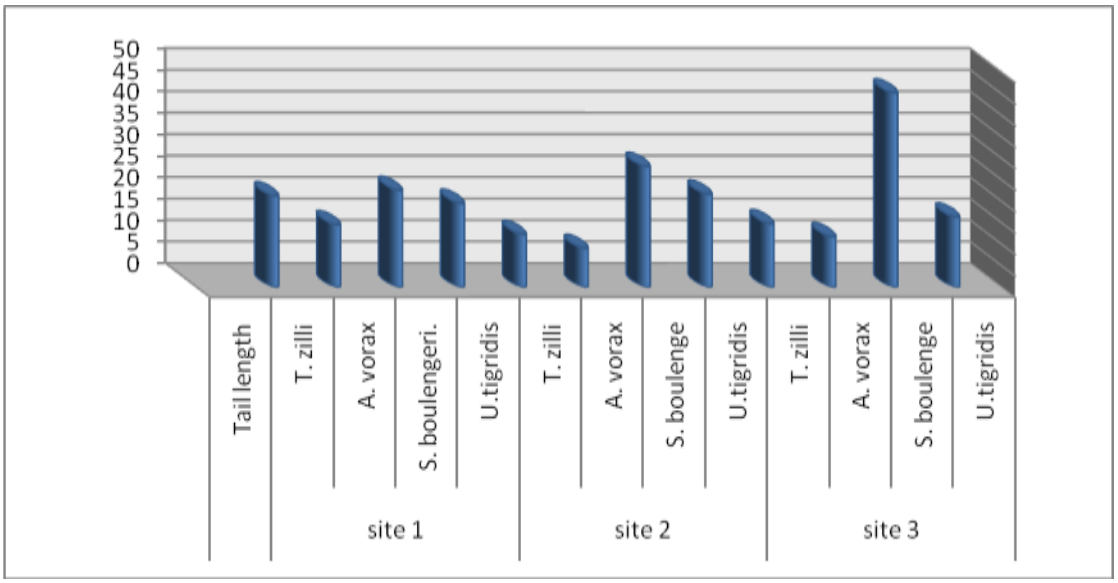


Figure(2) : Classes of DNA damage in studied *T. zilli* , *A.vorax*, *S. boulengeri* , *U.tigridis* according to Comet assay. Class1: Low damage, Class 2: Medium damage, Class 3: High Damage 40 X)

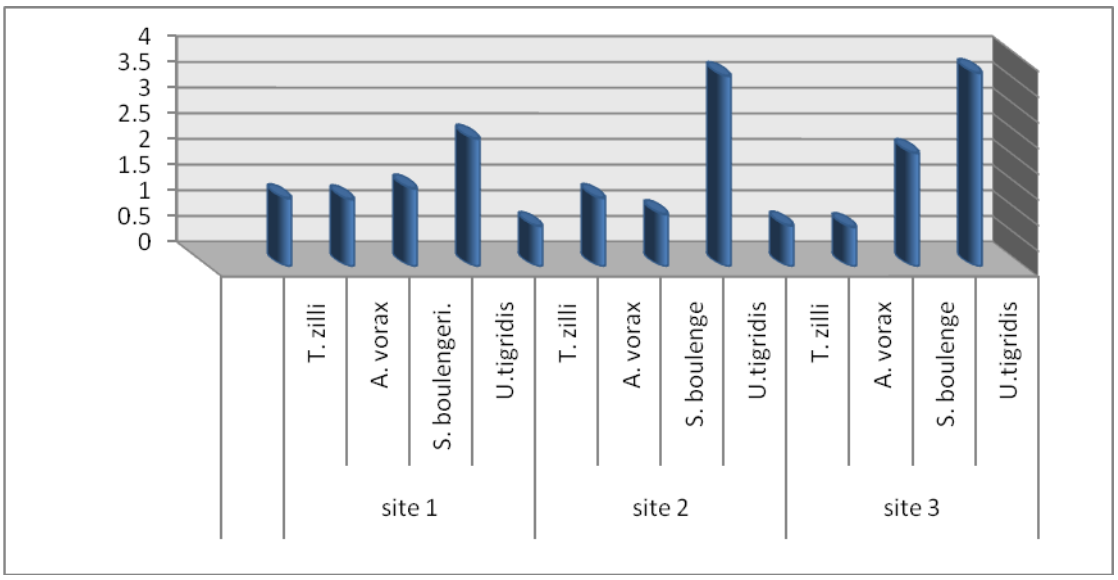
Table(1): The criteria of DNA damage in the species (*T. zilli, A.vorax, S. boulengeri , U. tigridis*) according to Comet Assay in the site 1-2-3 , March 2015 Mean±SD

N.Site / Species	Site (1)			Site(2)			Site(3)		
T.zilli	Damage 42	Comet L.	50.± 0.01	Damage 42	Comet L.	22.2± 1	Damage 43	Comet L.	34 .3± 3
		Tail Lμm.	21.± 1		Tail Lμm	12.2± 2		Tail Lμm	14.7± 2
		Moment %.	1.36 0.01		Momen t%	0.78± 0.02		Momen t%	0.79± 0.3
	Control 8	Comet L.	64± 4	8	Comet L.	42.3± 2	Control 7	Comet L.	756± 2
		Tail Lμm.	8.8± 1		Tail Lμm	9 ± 1		Tail Lμm	7.8± 3
		Moment %	1.3± 1		Momen t%	0		Momen t%	1.2± 2
A.vora	Damage 35	Comet L.	35.± 0.01	Damage 36	Comet L.	37.6± 2	Damage 36	Comet L.	65.4± 2
		Tail Lμm.	14.4± 2		Tail Lμm	16.5± 3		Tail Lμm	11.7± 3
		Moment %	1.3± 2		Momen t%	1.3± 1		Momen t%	0.76± 0.03
	Control 15	Comet L.	47.4± 0.01	Control 14	Comet L.	58.2± 19	Control 15	Comet L.	65 ± 4
		Tail Lμm.	7.7± 0.02		Tail Lμm	15 ± 3		Tail Lμm	12 ± 1
		Moment %	0.0		Momen t%	0		Momen t%	0
S. boulengeri	Damage 43	Comet L.	48.2± 0.01	Damage 48	Comet L.	53.8± 3	Damage 41	Comet L.	17.8± 2
		Tail Lμm.	22.5± 0.4		Tail Lμm	28.0± 2		Tail Lμm	45.2± 2
		Moment %	1.5± 0.03		Momen t%	1.0± 1		Momen t%	2.20± 0.1
	Control 7	Comet L.	65.1± 0.1	Control 2	Comet L.	44± 4	Control 9	Comet L.	12.3± 3
		Tail Lμm.	40.0± 4		Tail Lμm	17,5± 3		Tail Lμm	50.3± 2
		Moment %	0± 0		Momen t%	0		Momen t%	0
U.tigridis	Damage 41	Comet L.	61.9± 2.0	Damage 41	Comet L.	61.7± 2	Damage 36	Comet L.	56.4± 1
		Tail Lμm.	19.5± 3		Tail Lμm	21.6± 3		Tail Lμm	16.3± 2
		Moment %	2.4± 1		Momen t%	3.70± 0.1		Momen t%	3.7± 2
	Control 9	Comet L.	56.2± 3	Control 9	Comet L.	60.2± 0.1	Control 14	Comet L.	50.5± 2
		Tail Lμm.	30± 3		Tail Lμm .	13.5± 3		Tail Lμm .	5.5± 2
		Moment %	0 ±0		Momen t%	0		Momen t%	0

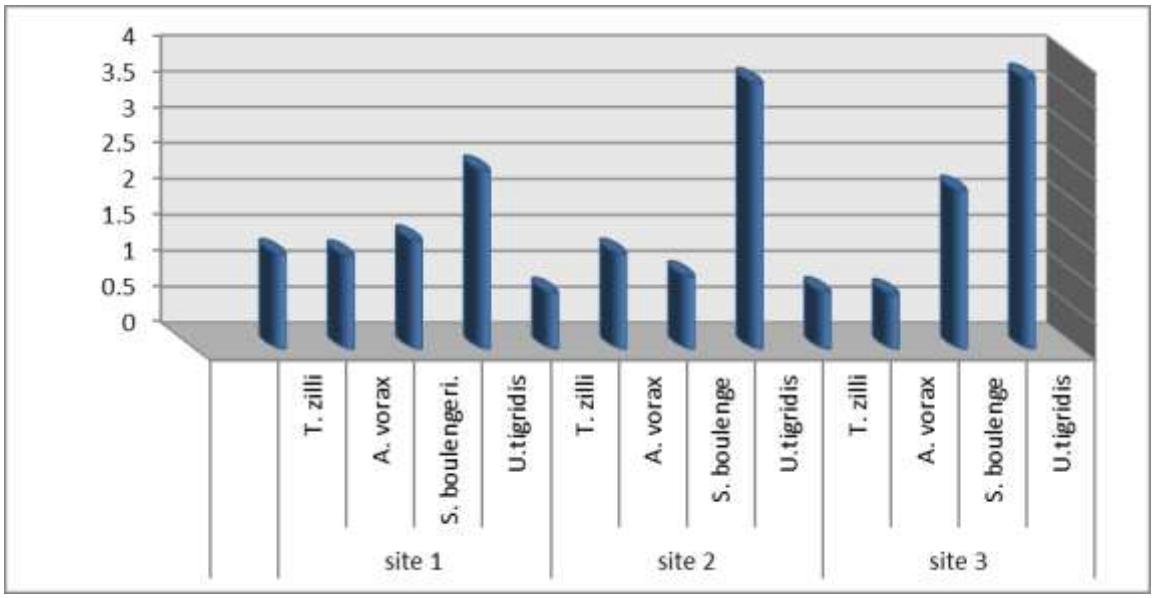
In the (Figure:3-4-5),which refer to measurements Tail length μm as indication of DNA damage, it has the highest level in st.1 in the species *S. boulengeri , T. zilli,U. tigridis* at respectively, and the highest level of DNA damage in the st.2 in the species *S. boulengeri, U. tigridis A. vorax* at respectively, and it highest level in the st.3 in species *S. boulengeri*



Figure(3):Variations of Tail length (µm) in *(T. zilli , A. vorax , S.boulengeri , U.tigridis*) in 1-2-3 site according to Comet Assay



Figure(4): Variations of comet length in *(T. zilli , A. vorax , S.boulengeri , U.tigridis*) in 1-2-3 site according to Comet Assay



Figure(5):Variation DNA moment % in T. zilli , A. vorax , S.boulengeri , U.tigridis in 1-2-3 site according to Comet Assay

Discussion:

DNA extraction has been proposed as auseful parameter for assessing the genotoxicpropereties of environmental pollutants many of these pollutant are chemical carcinogens and mutagens with the capacity to cause various type of DNA damage (Khalid et al.,2006) , and generation of ROS which causes a lot of damage and inducation of DNA damage (An et al.,2012)

Explained the case of DNA is highly fragmented due to the response to the state of pollution in those sites and the status of the damage that made clear in (Comet assay) within the index the (Tail moment µm) and index (Tail length µm) in this studying the studying coincided with (Shaw et al.,2004)

According to the results, which clarified the table Comet assay and through the values and indicators of damage (Comet lengthµm,Taillengthµm ,Tail Moment µm) and control (Comet length,Taillength,Moment)

Comet length is positively coreelated with the level DNA breakage in cell(Sinch et al.,1988) because the distributionthe comet was heterogenous ,histogram were applied to display information,the mean value of the comet length in aparticular sample was taken asan index of DNA damage in this sample

And that the ratio damage highest in species S.boulengeri. it means that the species of environmental did not resist and that environment stress in those sites and led to the damage because of the high proportion of damage cells means an increase in contaminated dose (Ome,1996) , This may be due to either their inability to continue to secrete enzymes to resist contaminated damage in those sites and this is indicated by the results of the enzymes, as was the lower rate and less positive impact and difference moral was that species in those sites may be due to the increase continued exposure to contaminated then change the resistance enzymes to reach the damage DNA.(Chow,2002). Has indicated the results of the Comet assay that the second site, which referred to the results of enzymes being more excretion sites of enzymes as the most vulnerable sites of pollutants through the case of the rate damage to the species that showed a lack of concentration and effectiveness of the enzymes, which all come as a reaction of free radicals and to the fact

that,the free radical which escape antioxidant suppression many find their way across nuclear membrane indicating DNA strand breakage (Georg,2014)

The following are the T.zilli,U.tigridis,A.vorax respectively, but a clear contrast the st.2 is less damage of the first and third site of the rate and the second reason is due to the rate resistance shown by species T.zilli,U.tigridis,A.vorax in response to persistent was through the rate and the number and concentration of the supreme differences moral positive in that site made there is an opportunity to resist the damage, unless the exposure of the damage and the concentration of contaminated rate increased as indicated several studies on DNA damage emploing the comet assay performed on fish and mollusca revealed a direct effect of exposure to different (Lima et al.,2006.(

Surrounding the circumstances of the last factors that make the organism less resistant conditions even with excretion of the enzyme and its resistance to environmental stresses, including the nature of life organism itself for the time of the month and year, and reproduction, the time of exposure to contaminated even if it is less migration from the site of a to another site in the water column.

Sometimes it might be for some species of fish the ability to repair damage to the DNA (Vinodhini,2009). The movement sometimes active species more vulnerable to pollution and heavy elements and exposed to factors that damage DNA and it is evident in the species S. boulengeri who in the sediment and stays for a long time and is transmitted to the water column it is exposed to the types of pollutants (Salman et al.,2010).So as well as for the movement of fish order to species fish for it activity (Salman et al.,2012)

References:

AnnHauser,R.;Calixtodecampos,R.;Lourencoziolli,R.(2012).Fish Metalloprotiens as Biomarker of Environmental, contamination Reviews of Environmental contamination and toxicology . springer science (218) (2012)

Black ,M.C.; Ferrell ,J.R.,Horning ,R.C.&Martin,L.K.(1996). DNA strand breakage in fresh water mussels (Adontagrandis) exposed to lead in the laboratory and field Environmental Toxicology and chemistry, 15(5),802-808.

Chow C ; Hong C.(2002) . Dietary Vitamin E and selenium and toxicity of nitrite and nitrate toxicology 180 :195- 207

Feder ,M.E., Hoffman ,G.E.,(1999). Heat-shock protein ,Molecular chaperones , and the stress response : evolutionary and ecological physiology .Annu.Rev. physiol, (61)

Georg , O. O.; Amaeze, N. H.; Soghanmu, T. O.,Otitoloju , A.A.(2014). Biomarker Responses in TympanotousFuscatusVar Radula (L) Inhabiting an Oil –Impacted and Fire –Ravaged Mangrove Ecosystem , current Advances in Environmental science ,American V- king scienctific publishing , (2), pp:101-111.

.Lima,P.L.; Benassi. J.c.;Pedrosa ,R.C.;Dalmagro,J. ; Oliveira , T.B. (2006). Time –course Variations of DNA damage and Biomarker of oxidative stress in Tilabia (Oreochromisniloticus) exposed to effluents from aswine Industry .Archives of environmental contamination and toxicology.(50)23-30(2006).

Obiakar ,M.O.; Okonkwo J.C.; Nnabude P.C.;Ezeonyejiaku, C.D.(2012) .Eco-genotoxicology : Micronucleus Assay in fish Erythrocytes as In situ Aquatic Pollution Biomarker . ,journal of Animal science Advances , 2(1): 123-133. .

Salman , J.M. Salaa,M.M. and Hassan,F.M.(2010) . Environmental study use some aquatic corganism as bioindicators to heavy metals pollution in Euphrates river Iraqi j. of market research and consumer production (2) No.3.

Salman ,J. M.; Al-mamoori;A. M.J.;AL-saddi,A. H.; AL-Terchi , M. N.AL.(2012). DNA damage in common carp fish (Cyprenuscarpio L.) Induced by acute Exposure to copper &cadmium,world Applied science journal 20(5) : 679-682.

Vinodhini ,R.andNarayanar, M. .(2009). Biology changes of antioxidant enzyme in common Carp (Cyprinuscarpio L.) after heavy metal exposure, Anim. Sci., 33(4) : 273 -278 .