

The Systematic Approach of Heavy Metals Accumulation in *Oreochromis niloticus* from Theroor Wetland, Kanyakumari, India

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Received 25 January 2020; Accepted 13 March 2020; Published on 4 April 2020

ABSTRACT

Exposure to heavy metals is an important environmental problem resulting from anthropogenic activities. So, in the present study, the bioaccumulation of heavy metals (Pb, Cd, and Cu) in the muscles of fresh water edible fish *Oreochromis niloticus* caught from the Theroor wetland was carried out as they remain present in some or the other form harmful for the human body and its proper functioning and to ascertain its impact on the protein levels in muscle of

selected fish *Oreochromis niloticus*. The results of the current study indicated that heavy metals in the edible parts of the investigated fish are not in the permissible safety levels for human consumption (FAO/WHO). Thus, this paper gives an overview of the manipulation of fish as a biomarker of heavy metals through proteomic studies which have proven to be very useful in the environmental pollution monitoring.

Keywords Heavy metals, Protein, Muscle, *Oreochromis niloticus*.

INTRODUCTION

Culture fisheries (marine and fresh water) are gaining additional emphasis due to our concern is sustainability, greener solutions, conservation and food security. Recent days fresh water fishes has been recognized as an excellent food source for human beings and is preferred as a perfect diet not only due to its excellent taste and high digestibility but also because of the presences of unsaturated fatty acids, essential amino acids, minerals for the formation of functional and structural proteins (Kumar et al. 1992) and also due to the significant positive impact it brings in improving the quality of dietary protein by complementing the essential amino acids that are often present in low quantities in vegetable based diets (Sargent et al. 1995). The fresh water bodies, the habitat of fresh water fishes are according to the current scenario getting very much highly polluted due to unawareness of rural and urban people intervention and activities. So being the major aquatic organism and being accumulated to

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all the zones of living medium they respond to a wide range of natural and man-made environmental stressors, which can lead to molecular changes within their tissues especially muscles which contribute about 34–48% of the total body mass in fish.

As of today, consumers are more aware to know about the food they eat and also, they have so many methods to get clarified. Currently proteomics a bioinformatics tool plays an increasing role in food authentication. Fish, the globally accepted cheapest food but with rich source of protein, minerals and vitamins was used by all people. But they are in need of food in the farm as well as better water quality parameters. Moreover, the key aspect of aquaculture is characterization of the muscle proteome as muscle plays a central role in whole-body protein metabolism, encompassing physiology and growth. Since proteins can be used as bio molecular tool for many food property analyses like food safety, food authentication, food quality, traceability, shelf-life, recently many molecular biologists started focusing on proteomic works in relation to environmental stress on fish to determine, evaluate and improve of the fish species production and management. Bostock et al. (2010) in his view stated that while aquaculture industry is growing at a higher rate than any other animal food-producing sector nutrition, welfare and health management have been thought to be the main limitations to an efficient production in aquaculture systems.

Thus, computational study will allow muscle proteins to be studied at greater level in detail using a variety of bioinformatics tools that are available for making detailed comparative study and visualization of amino acid sequences, which provides knowledge about molecular evolution and variety of information related to structure and function of protein. Besides adding to the existing knowledge base on comparative muscle proteomics, the information generated would also serve as the baseline proteogenomic information on this exotic species *Oreochromis niloticus* the fresh water fish of the family : Cichlidae inhabiting in the tropical wetland water bodies of Indian subcontinent, is an important food fish with high nutritive value. This species and is also considered as a species of choice in wetland pollution monitoring because it

is a commercially important species contributing a major share to fresh water aquaculture production in the Indian subcontinent; however, little omics information is available on this species. So, the main aim of this present study was to examine the changes in the muscle protein expression profiles of common fresh water fish *Oreochromis niloticus*, of Theroor wetland (Ohlendieck 2011).

MATERIALS AND METHODS

The proteins and enzymes in *Oreochromis niloticus* that are responsible for heavy metal assimilation were identified at the genome level. The proteins with already known function were explored by text mining whereas the functions of hypothetical and uncharacterized proteins were predicted. The KEGG Orthology (KO) system is a pathway based definition of orthologous genes (Moriya et al. 2007, Craig 2005). The function of missing proteins was predicted according to the methods described by Chellapandi (2011). Briefly, a protein whose function was not yet reported in databases or annotated function not compiled in KEGG pathway database, but which has separate KO system in the KEGG GENES database, their protein function was assigned to be predicted with KO.

Proteome retrieval

The complete proteome of *Oreochromis niloticus* was retrieved from Uniprot Knowledgebase. Uniprot KB/Swiss-Prot is the manually annotated and reviewed section of the Uniprot Knowledgebase (Uniprot KB) (The Uniprot Consortium).

Metabolic pathway analysis

The metabolic pathway analysis of *Oreochromis niloticus* was carried out using KEGG (Kyoto Encyclopedia of Genes and Genome). KEGG is a database resource that integrates genomic, chemical and systemic functional information. Further, protein-protein interactions of *Oreochromis niloticus* can be identified using STING database (Szklarczyk et al. 2015).

Cell simulation

Simulation of intervention of heavy metals in the

Table 1. Proteins involved in lead assimilation in *Oreochromis niloticus*.

Sl. No.	Uniprot ID	Protein	Gene name	Reaction	Function
1	13J4P3	Lactoyl glutathione lyase	Glo1	(R)-S-lactoyl glutathione = glutathione + methylglyoxal	Catalyzes the conversion of hemimercaptal, formed from methylglyoxal and glutathione, to S-lactoyl glutathione
2	13KGY7	S-formyl glutathione hydrolase	Esd	S-formyl glutathione + H ₂ O = glutathione + formate	Serine hydrolase involved in the detoxification of formaldehyde
3	131XM8	S-(hydroxy methyl) glutathione dehydrogenase	LOC100695823	S-(hydroxy methyl) glutathione + NAD (P) ⁺ = S-formyl glutathione + NAD (P) H	S-(hydroxymethyl) glutathione dehydrogenase activity
4	13K4Z5	Glutathione S-transferase	gstk1	RX + glutathione = HX + R-S-glutathione	Glutathione transferase activity
5	13KTP3	Glutathione peroxidase	-	-	Glutathione peroxidase activity; response to oxidative stress

system of *Oreochromis niloticus* was carried out using cell designer software (Funahashi et al. 2008). It is an application in which compartmental topology, geometry, molecular characteristics and relevant interaction parameters can be used defined. It automatically forms a corresponding mathematical system of ordinary and/or partial differential equations from the given biological description.

RESULTS

The extensive text mining with the available databases resulted in the identification of the proteins involved in the assimilation of heavy metals in *Oreochromis niloticus*.

Lead assimilation system

A total of 5 proteins were identified in *Oreochromis niloticus* involved in lead assimilation. The list of proteins is tabulated in Table 1, which signifies genes such as glo 1, esd, Loc100695823, gstk 1 and unknown corresponding to proteins lactoyl glutathione lyase, S-formyl glutathione hydrolase, S-(hydroxy methyl) glutathione dehydrogenase, glutathione S-transferase and glutathione peroxidase respectively. The KO assignment for these proteins reveals its role in glutathione metabolism which is portrayed in Fig. 1. Further, the rates of metabolic changes of the selected proteins in the presence lead at various concentrations were simulated using cell designer and the results are presented in Table 2. From there we can be in-

ferred that the concentration of the proteins lactoyl glutathione lyase (13J4P3), S-(hydroxy methyl) glutathione dehydrogenase (131XM8), glutathione S-transferase (13K4Z5) increases with increase in the concentrations of lead. On the other hand, the concentration of the proteins, S-formyl glutathione hydrolase (13KGY7) and glutathione peroxidase (13KTP3) decreases with increasing concentration of lead at various time intervals.

Cadmium assimilation system

A total of 5 proteins were identified in *Oreochromis niloticus* involved in cadmium assimilation. The list of proteins is tabulated in Table 3. Which

Table 2. Rate of change of lead assimilation system at various concentrations of lead in *Oreochromis niloticus*.

Conc of lead	Concentration in mM					Time in min
	13J4P3	13KGY7	131XM8	13K4Z5	13KTP3	
2.1	50	25	70	80	15	5
2	45	32	60	75	22	10
1.9	42	35	52	45	25	15
1.7	33	42	45	35	32	20
1.4	29	45	40	30	35	25
1.2	23	50	35	25	40	30
0.9	20	55	30	20	45	35
0.8	15	60	20	10	50	40
0.7	0	70	10	5	60	45
1.9	42	35	52	45	25	50
1.8	35	40	48	48	30	55
1.7	33	42	45	35	32	60
Correla- tion	0.961956	-0.97014	0.961119	0.903499	-0.97014	

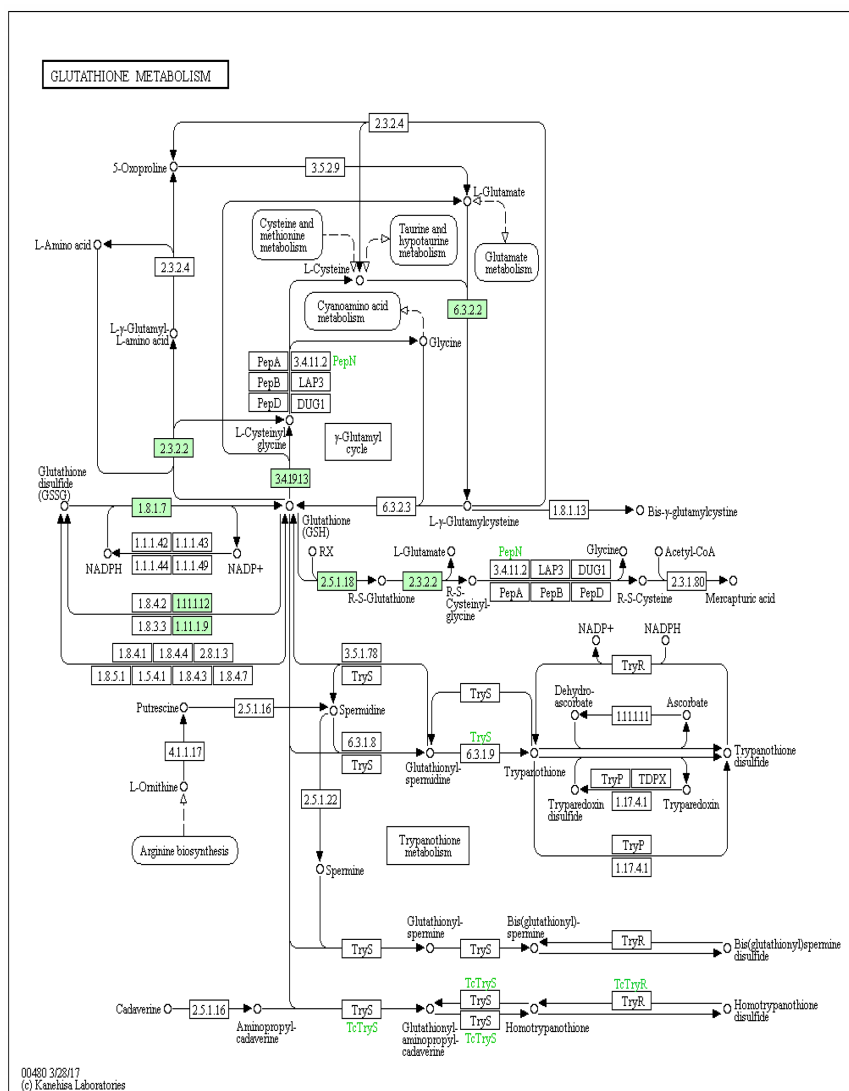


Fig. 1. Network of glutathione metabolism in *Oreochromis niloticus*.

signifies genes such as *Loc100696572*, *Jun*, *Rad 51* and unknown corresponding to all uncharacterized proteins. Superoxide dismutase was also involved in cadmium assimilation with gene name *Sod 2*. The KO assignment for these proteins reveals its role in apoptosis which is portrayed in Fig. 2. Further, the retes of metabolic changes of the selected proteins in the presence of cadmium at various concentrations were simulated using cell designer and the results are presented in Table 4. From Table 4 it can be inferred that the concentration of the proteins 13KVU8,

13KYD0 and 131YF0 increases with increase in the concentrations of cadmium. On the other hand, the concentration of the proteins, 13K6D5 and 131ZK4 decreases with increasing concentration of cadmium at various time intervals.

Copper assimilation system

A total of 5 proteins were identified in *Oreochromis niloticus* involved in copper assimilation. The list of proteins is tabulated in Table 5 includes cop-

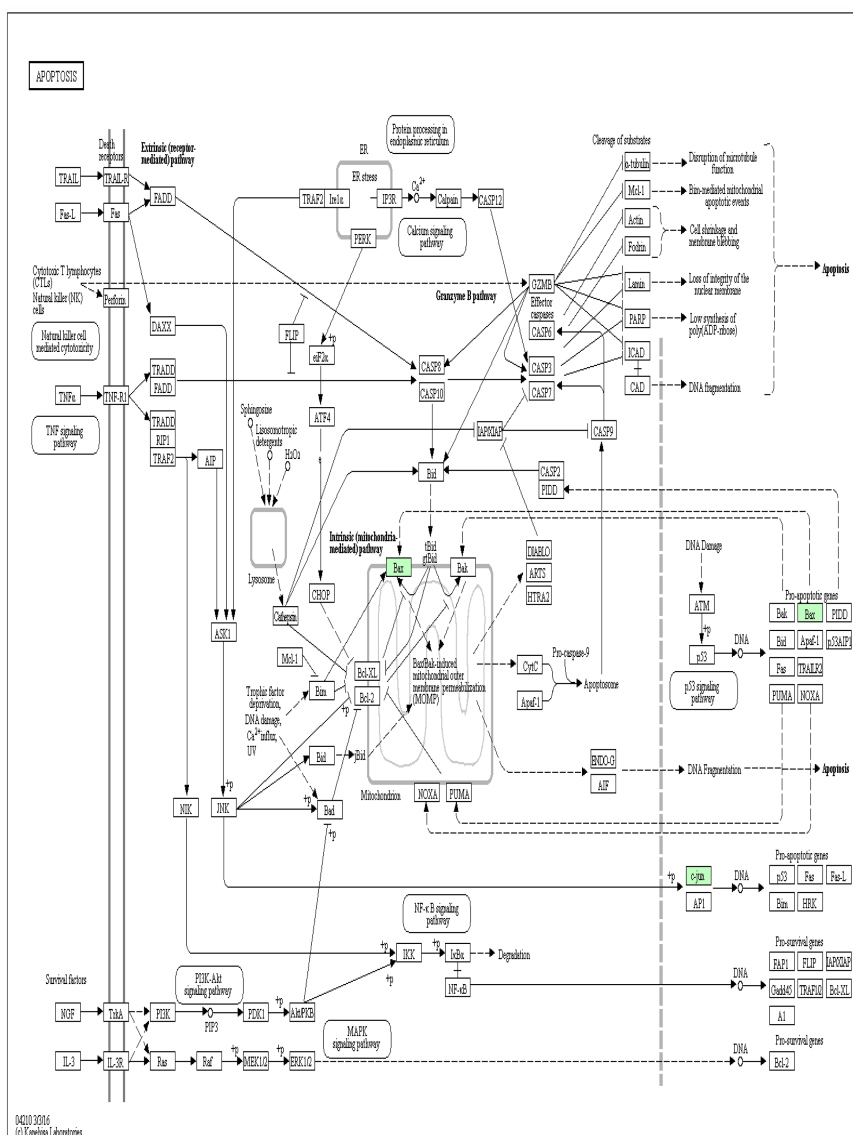


Fig. 2. Network of apoptosis in *Oreochromis niloticus*.

per-transporting ATPase, Cytochrome c oxidase, Amine oxidase, Superoxide dismutase (Cu-Zn) and Tyrosinase-related protein 1. The KO assignment for these proteins reveals its role in Tyrosine metabolism which is portrayed in Fig. 3. From Table 6, it can be inferred that the concentration of the proteins F5C7J6 and 13JAL8 exhibit positive correlation with the concentration of copper. But, the concentration of the proteins, D2Y6D3, 13K1J1 and Q61U22 decreases

with increasing concentration of copper at various time intervals.

DISCUSSION

Heavy metals occur naturally in the earth's crust at various levels. Recently due to latest technological and industrial developments many dangerous chemicals have been released without any pre-treatments

Table 3. Proteins involved in cadmium assimilation in *Oreochromis niloticus*.

Sl. No.	Uniprot ID	Protein	Gene name	Reaction	Function
1	13KVU8	Uncharacterized protein	LOC100696572		ATP Binding; response to cadmium ion superoxide dismutase activity; response to cadmium ion transcription factor activity;
2	13K6D5	Superoxide dismutase	Sod2	$2 \text{ superoxide} + 2 \text{H}^+ = \text{O}_2 + \text{H}_2\text{O}_2$	response to cadmium ion recombines activity; response to cadmium ion positive regulation of cysteine-type endopeptidase activity involved in apoptotic process;
3	13KYD0	Uncharacterized protein	JUN	-	response to cadmium ion
4	131ZK4	Uncharacterized protein	Rad51	-	
5	131YF0	Uncharacterized protein	-	-	

directly are indirectly into inland water bodies. Nonetheless, the problems arises when they are released at chronic levels into the environment due to rapid urbanization, anthropogenic activities, modern agricultural practices, using huge amount of pesticides and chemicals in lands in and around water bodies, getting contaminated with heavy metals due to over use of municipal waste, compost waste, influx of heavy metals from industries all together resulting in accumulation of heavy metals in the environment, which in turn causes toxicity to living organisms in particular- fish. This current situation has further got worsened by increasing population growth leading to inherent food demand specifically protein rich food i.e. fish.

As of today the consumption of fish, the poor man's lobster by human beings have increased globally to 80-90% because, biologically fish muscle proteins contain all essential nutrients, so fish holds prime importance in food industry. Furthermore, it is very nutritious part of human diet because it is rich in vitamins, minerals and all essential amino acids in right proportion. In addition, fish populations tend to be stable and easy to collect and they were widely used in bio monitoring of environmental pollutants, heavy metals in specific. So, heavy metals contamination of water causing stress to organisms has become one of the important constraints to fish productivity and fish food quality (protein). Moreover majority of human population especially rural people living within the surrounding of fresh water bodies which often acts as sinks for such pollutants arising from anthropogenic materials in environment in unprecedented levels consume fish harvested from them.

Xenobiotics (i.e.) heavy metals bio accumulated in aquatic inhabitants are subsequently transferred to humans through the food chain. The movement of toxicants through a transported medium may significantly impact all immune organisms i.e. fauna, flora as populations or as ecological communities. Also a variety environmental process may alter the chemical structure of pollutants which could challenge organisms in particular locations by altering their community in a particular location and by influencing their ability to with stand further challenges posed by anthropogenic activities and pollutant. So, therefore very complex system called metal assimilation system was taken up to analyze the fish muscle protein using different computational tool as protein various in amount in polluted environment. So biomarkers with in sentinel organism (*Oreochromis niloticus*) have been extensively used to evaluate changes in

Table 4. Rate of change of cadmium assimilation system at various concentrations of cadmium in *Oreochromis niloticus*.

Conc of cadmium	Concentration in mM					Time in min
	13KVU8	13K6D5	13KYD0	131ZK4	131YF0	
2.05	70	5	89	5	56	5
2.04	65	12	76	7	54	10
2.05	68	15	65	5	58	15
0.92	63	19	54	12	59	20
0.85	61	25	48	15	49	25
0.79	55	36	42	13	47	30
0.71	43	42	37	14	39	35
0.62	39	56	33	25	37	40
0.53	35	63	29	27	34	45
1.03	50	77	25	32	29	50
1.02	51	89	21	35	24	55
1.03	52	95	19	37	15	60
Correlation	0.807288	-0.55473	0.7945	-0.57952	0.507741	

Table 5. Proteins involved in copper assimilation in *Oreochromis niloticus*.

Sl. No.	Uniprot ID	Protein	Gene name	Reaction	Function
1	F5C7J6	Copper-transporting ATPase 1	-	-	Copper-exporting ATPase activity
2	D2Y6D3	Cytochrome c oxidase	-	-	-
3	13K1J1	Amine oxidase	LOC100705916	-	Copper ion binding
4	13JAL8	Superoxide dismutase (Cu-Zn)	Sodi 1	2 superoxide + 2 H ⁺ = O ₂ + H ₂ O ₂	Binds 1 copper ion per subunit
5	Q6IUZ2	Tyrosinase-related protein 1	-	-	Copper ion binding

the environment and habitat.

According to Liu et al. (2013) proteomics is a well-established technique in the post-genomic era, which deals with large scale expression of proteins in an organism. This being a powerful tool not only describes complete protein changes in any organisms but also helps in comparing variations in protein profile of an organisms at organ, tissue, cell and organelle levels under different stress condition including heavy metals Ge and Schekman (2013) too stated that omicstechnology, a robust molecular biomarker measurement of protein would allow early detection of environmental stress upon exposure of chemicals. It is also well known that proteins are the important bio molecule that directly takes part in any organism's stress response and moreover organisms adapting to heavy metals stress are always accompanied with proteomic changes. Thus proteomic analyses offer a new platform for identifying target proteins which take part in heavy metals detoxification and in studying complex biological processes and interactions among the possible pathways that involves a network of proteins. Thus, proteomic technique has be exploited in this current chapter for deciphering the possible intervention between protein abundance and stress adaptation as it can contribute to better understanding of physiological mechanisms under heavy metal stress and further signaling cascade that leads to changes in the expression to large number of genes in metabolic profile under heavy metal toxicity.

So in the present work muscle protein of *Oreochromis niloticus* has been analyzed for intervention of metals which will be beneficial for humans in drug designing and maintain food care from consumer point of view. The heavy metals considered most

toxic and apparently most poisonous to fresh water life which are reviewed in the present study include zinc, copper, nickel, lead, cadmium and mercury. Fish and people are primarily exposed to heavy metals like lead, cadmium, zinc, nickel, copper and mercury by food ingestion and breathing. These heavy metals get accumulated in the muscles, bones, blood and fat. Neonates and young children are especially delicate to even low levels of heavy metals. As recently pointed out, in several areas of the fresh water wetlands, high concentrations of these elements are present in many types of commercial important fish, *Oreochromis niloticus* (WHO 2005, Erdoayrul and Ates 2006, Nduka et al. 2010, Abdel-Mohsien and Mahmoud 2015, Rodrigues et al. 2012, Desmond et al. 2007). Long term consumption of food stuff (i.e. fish) contaminated with metals may lead to the accumulation of toxic metals in several vital organs resulting in perturbation of biochemical processes, which may

Table 6. Rate of change of copper assimilation system at various concentrations of copper in *Oreochromis niloticus*.

Conc of copper	Concentration in mM					Time in min
	F5C7J6	D2Y6D3	13K1J1	13JAL8	Q6IUZ2	
3.28	75	25	4	65	18	5
3.21	71	36	6	59	26	10
3.30	69	42	7	57	34	15
2.47	67	49	9	56	45	20
2.42	63	57	13	55	57	25
2.31	54	68	15	43	63	30
2.19	49	74	18	41	69	35
2.09	38	81	19	39	71	40
1.9	34	89	25	35	75	45
3.09	23	91	31	27	81	50
2.97	21	93	35	23	85	55
2.89	18	90	40	19	90	60
Correlation 0.200492 -0.43849 -0.12811 0.19827 -0.43405						

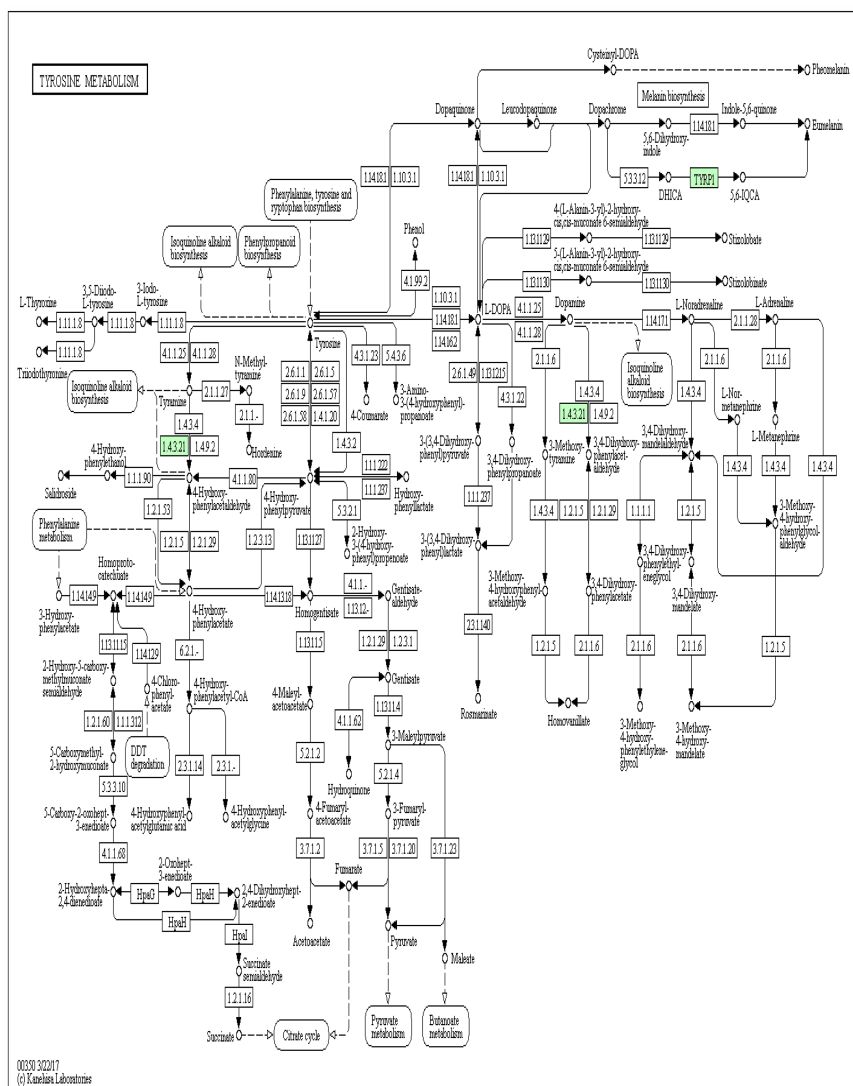


Fig. 3. Network of tyrosine metabolism in *Oreochromis niloticus*.

cause liver damage, kidney damage, cardiovascular problems, nervous problems, bone disorders, memory loss, mental retardation, low IQ, learning deficits in particular among young children and also in people of other age too.

The toxic effects of heavy metals can affect the individual growth rates, physiological functions, mortality and reproduction in fish (Amundsen et al. 1997, Mahmoud 2015). Lead deplete major antioxidants in the cell, especially thiol-containing antioxidants

and enzymes and can cause significant increases in a reactive oxygen species (ROS) production, followed by a situation known as “oxidative stress” leading to various dysfunctions in lipids, proteins and DNA (Ercal et al. 2001). This is further validated from the present study, that the lead assimilation system is a part of glutathione metabolism. Similarly, sub-lethal effects such as decreased growth, inhibited reproduction and population alterations may occur after chronic exposure to cadmium (Eisler 1985). This has been further proved from the present study

on the involvement of cadmium assimilation system with apoptotic pathway. On the other hand, copper forms a part of many enzymes and glycoprotein in fishes, it is important for nervous system function and is necessary for hemoglobin synthesis Sorensen (1991), Nordberg et al. (2007). In line with the findings, the present study confirms its role in tyrosine metabolism. In contrast, the present study reveals the role of nickel in calcium signaling pathway, it was already confirmed that nickel exposure results in decreased blood parameters in fishes (Gochfeld 2003). But mercury contamination causes irreversible damages, such as neurological impairment and lesions, behavioral and cognitive changes, ataxia, as well as convulsions, in addition to its harmful effect on reproduction which supports the present finding of the role of mercury assimilation system in cancer (Oliveira et al. 2006). In contrast, zinc exposure has been shown to induce histopathological alterations in ovarian tissue of *Tilapia nilotica* (degeneration and hyperaemia) (Abd and Gadir 1999) and liver tissue of *Oreochromis mossambicus*, but the present study shows its involvement in protein processing as it's a cofactor for many enzymes. Thus, the *In-silico* approach made in the present study revealed that fish proteins have functional significance and this study can be further taken up to next stage in exploring proteins of which can be incorporated in designing effective vaccines-drug designing approach.

The present study gives an overview of water quality, eco-biology of micro and macro organisms, the diversity status and impact of pollutants especially xenobiotic on fish biomolecules and their health effects on human beings. As pieces of continuity the next part of the present study was focused on protein molecule of *Oreochromis niloticus* because the proteome varies among organs with time and reflection of organisms to environment due to their adaptive nature. Because there is a general consensus about today's industrial and human activity impacts in the fresh water which is the cause of the depletion of a varied range of living organisms. It is well agreed with the present results that the current situation of wetland water seems to be further exacerbated by risk derived from the continuous incoming of industrial pollutant in form of heavy metals which in turn alters the biomolecules of the major aquatic life like

fish. So the analyses of the impact and its level on *Oreochromis niloticus* muscle proteomic analysis was carried out as it gives the snapshot of selected organisms state of being and map the entirety of its adaptive potential and mechanisms. On analyzing the impact of xenobiotics on fish muscle using system approach-bioinformatics tool resulted in the identification of the proteins involved in the assimilation of heavy metals in *Oreochromis niloticus*. There are 5 proteins involved in lead, cadmium, copper assimilation in *Oreochromis niloticus*; 4 proteins involved in zinc assimilation in *Oreochromis niloticus*; 3 proteins involved in nickel assimilation in *Oreochromis niloticus*; 1 protein involved in mercury assimilation in *Oreochromis niloticus* was identified using Uniport. Each and every heavy metal had its own impact on the biomolecule pathway like metal lead following the situation known as oxidative stress leads to various dysfunction in fish. Similarly, copper, nickel and mercury exposures resulted in decrease in blood parameter level and dysfunction of nervous system. Zinc exposure will induce histopathological. Finally, the function and status of the interested proteins are analyzed using Uniport, bioinformatics tool and discussed to obtain a better understanding of the molecular mechanisms in combination with other investigations. Most of the metals are present in edible portion of fish. Humans are also affected by eating fish and can cause a few of health problems. The levels of toxic elements in different fishes depend on the fish sex, age, season and place. The pollution of waterways with anthropogenic activities are the major cause of aquatic loss and imbalanced food chain. To eliminate and avoid the aquatic life loss there is need to use the advanced technologies generating less heavy metal pollution to environment.

CONCLUSION

Thus, Uniport being a very promising application in search and detection of different proteins and the characterization of biologically active proteins and the impact of contaminants in aquatic organisms have been applied in the present study to enhance the aquaculture proteomics for the global analysis of protein expression in fresh water fishes of commercial importance for the benefit of consumers. Form consumer point of view regarding the food quality

and health assurance aquacultural industries inspite of following traditional methods many different instrumental techniques have been proposed for food authentication one such new approach called proteomics has been applied to analyze the muscle composition of *Oreochromis niloticus* of Theroor wetland for creating awareness and database for future generations to conserve the natural resources like wetland ecosystem and their components especially fresh water fishes and their feeds. The present results also proved that, the fishes occupying high trophic levels, based on the higher levels of metal bioaccumulation, could be unsafe for human consumption so, periodic monitoring of heavy metals in fishes to ensure continuous safety of people in the area is recommended.

ACKNOWLEDGEMENT

Authors are thankful to the Head, PG Research Department of Zoology and authorities of Nirmala College for Women, Coimbatore for the facilities provided. One of the authors (SDK) thanks the UGC Govt of India for providing post-doctoral Fellowship (RefNo. F/31-1/2017/PDFSS-2017-18-TAM-13681 dated 19.06.2017).

REFERENCES

- Abd El, Gadir A (1999) Replacement of cereal energy by molasses at increasing protein/energy ratio for fattening rabbitlets. Thesis MSc, University of Khartoum.
- Abdel-Mohsien HS, Mahmoud MA (2015) Accumulation of some heavy metals in *Oreochromis niloticus* from the Nile in Egypt: Potential hazards to fish and consumers. *J Environ Prot* 6 : 1003—1013.
- Amundsen PA, Staldvik FJ, Lukin AA, Kashulin NA, Popova OA, Reshetnikov YS (1997) Heavy metal contamination in fresh water fish from the border region between Norway and Russia *Sci* 201 : 211—224.
- Bostock J, Mcandrew Richards R, Jauncey K, Telfer T, Lorenzen K, Little D, Ross L, Handisyde N, Gatward I, Corner R (2010) Aquaculture : Global status and trends. *Philos Trans R Soc Lond B Biol Sci* 3 : 2897—2912.
- Chellapandi (2011) Molecular evolution of methanogens based on their metabolic facets. *Front Biol* 66 : 490—503.
- Craig IW (2005) The role of monoamine oxidase a MAOA in the aetiology of antisocial behavior: The importance of gene-environment interactions. *Novartis Found Symp* 268 : 227—237.
- Desmond Doran, Alex Hill, Ki-Soon Hwang, Gregoire, Jacob (2007) Supply chain modularization cases from the French automobile industry. *Int J Prod Econ* 106: 2—11.
- Eisler R (1985) Cadmium hazards to fish wildlife and invertebrates: A Synoptic Review US Fish and Wildlife Service Report 85 (1.2) Washington DC.
- Ercal N, Gurer-Orhan H, Aykin-Burns N (2001) Toxic metals and oxidative stress part 1 : Mechanisms involved in Metal induced oxidative damage 1 (6) : In press.
- Erdoayrul A, Ates D (2006) Determination of cadmium and copper in fish samples from Sir Dam Lake and Menzelet Dam Lake Kahramanmaras Turkey. *Environ Mon Ass* 117 : 281—290.
- Funahashi S, Hasegawa T, Nagano A, Sato K (2008) Differential expression patterns of messenger RNAs encoding Nogo receptors and their Ligands in the rat central nervous system. *J Comp Neur* 506 : 141—160.
- Ge L, Schekman R (2013) The ER-Golgi intermediate compartment feeds the phagophore membrane. *Autophagy* 10 : 170—172.
- Gochfeld M (2003) Cases of mercury exposure bioavailability and absorption. *Ecol Environ Safet* 56 : 174—179.
- Kumar D, Karim AMA, Nandi SB (1992) Fish culture manual: Institutional strengthening in the fisheries sector, Bangladesh. Project Reports 32, FI/FIRA, Dacca, Bangladesh, pp 49.
- Liu J, Huang X, Withers BR, Blalock E, Liu K, Dickson RC (2013) Reducing sphingo lipid synthesis orchestrates global changes to extend yeast lifespan. *Aging Cell* 12 : 833—841.
- Mahmoud Manal (2015) Health risk assessment of heavy metals for egyptian population via consumption of poultry edibles. *Adv Anim Vet Sci* 3 : 58—70.
- Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M (2007) KAAAS : An automatic genome annotation and pathway reconstruction server. *Nucleic Acids Res* 35 : W182-W185.
- Nduka JK, Orisakwe OE, Okerulu IO (2010) Heavy metal levels in muscles of some fish species from Aladja river warri Nigeria : A public health concern. *Adv Environ Biol* 4 : 125—130.
- Nordberg GF, Nogawa K, Nordberg M, Friedmann JM, Cadmium, Nordberg GF, Fowler BA, Nordberg M (2007) Friberg L (ed). Handbook on the Toxicology of Metals Amsterdam : Elsevier, pp 445—486.
- Ohlendieck K (2011) Skeletal muscle proteomics: Current approaches technical challenges and emerging techniques. *Skelet Muscle* 1 (6) : In press.
- Oliveira PH, Batagov A, Ward J, Baganz F, Krabben P (2006) Identification of erythroblastin, a hydroxamate-type siderophore produced by *Saccharopolysporaerythraea*. *Letet Appl Microbiol* 42 : 375—380.
- Rodrigues FS, Doughton G, Yang B, Kelsh RN (2012) A novel transgenic line using the Cre-lox system to allow permanent lineage labelling of the zebrafish neural crest. *Genesis* 50 : 750—775.
- Sargent JR, Bromage NR, Roberts RJ (1995) Origin and functions of eggs lipids : Nutritional implications and broodstock management and egg and larval quality . Blackwell Sci London, pp 353—372.
- Sorensen EMB (1991) Metal Poisoning in Fish CRC Press Boca Raton.
- Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Kuhn M (2015) STRING v10: Protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res* 43 : D447-D452.
- World Health Organization (2005) Public Health Agency of Canada. Preventing chronic diseases : A vital investment. Geneva, World Health Organization.