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Study of Combined Impact of *Chlorella Vulgaris* And *Spirulina Platensis* on Sub - Lethal & Lethal Concentrations of Copper & Zinc Toxicity on *Labeo Rohita (Ham), Clarias Batrachus (Linn)* And *Channa Punctatus (Bloch).*

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ABSTRACT: The Chlorella vulgaris and Spirulina platensis influenced the sub-lethal & lethal effect of copper & zinc caused variations in brain compartmentation (cerebrum, diencephalons, cerebellum & medulla oblongata) of hexokinase in Labeo rohita, Clarias batrachus & Channa punctatus under acute or short term exposure. The sub-lethal and lethal levels of copper & zinc inhibited the hexokinase to a highest extent in diencephalon than in cerebrum, medulla oblongata & cerebellum in Labeo rohita in comparison to Clarias batrachus & Channa punctatus but lesser than the fall of the hexokinase enzymes in the above said fish species directly exposed to sub-lethal & lethal levels of copper & zinc directly without Chlorella vulgaris and Spirulina platensis compelled us to develop an insight to understand the positive impact on important bio-chemical parameters like enzymes that are important to promote a variety of anabolic & catabolic processes in an organism effectively reflects that microbes act as antidote effect fall heavy metal toxicity and the less fall of the hexokinase enzyme under investigation may be that Chlorella vulgaris and Spirulina platensis has a soothing impact and hence the Chlorella vulgaris and Spirulina platensis are able to decrease the sub-lethal & lethal toxicity of sub-lethal & lethal levels of copper & zinc.
The following finding may help to understand the microbe-metal interaction and sub sequent detoxification of the metal caused toxicity to a less extent in a better way.

Key Words:- Chlorella vulgaris , Spirulina platensis, Copper, Zinc hexokinase**, Labeo rohita, Clarias batrachus , Channa punctatus.**

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I. INTRODUCTION

Heavy metals are dangerous because they tend to bioaccumulation *[Bano et al., 2007 & Aniko et al., 2015]*. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted *[Sharma & Sharma, 2005;Shaffi & Kakaria,2006; Manjrekar et. al., 2008; Ansari & Bhandari, 2008; Kaur & Bansal, 2008; Manousaki & Kalogerakis, 2009 & Murali & Mehar, 2014 & Nichat, 2018]*.

In the present investigation the author made an attempt to investigate the combined influence of *Chlorella vulgaris* and *Spirulina Platensis* on sub-lethal & lethal concentration of metal (Cu & Zn) caused enzymatic variations *hexokinase* in different brain regions [cerebrum, diencephalons, cerebellum & medulla oblongata] of fresh water teleosts i.e. *Labeo rohita* (Ham.), *Clarias batrachus* (Linn.) and *Channa punctatus* (Bloch.) on a comparative basis from a tropical environment under short term exposure (acute studies).

II. MATERIAL AND METHODS

Alive, healthy, mature, disease-free & active *Labeo rohita* (Ham.), *Clarias batrachus*(Linn.) and *Channa punctatus*(Bloch.) 120-130 gm. of 18-20 cm. (standard length) were obtained from few selected local ponds to avoid ecological variation and acclimatized in the laboratory condition for a period of seven days and were subjected for various exposures and investigations.

Determination of safety, Sub-lethal and lethal concentration: Safety, sub-lethal concentrations of copper was determined on *Labeo rohita,Clarias batrachus* and *Channa punctatus* by the *Probit Analysis Method*

[Finney,1971]. Higher concentration of copper was used and slowly reduced the amount of concentration to know the Lc 50/100 value for 96-hour exposure.

Acute studies: The *Labeo rohita* , *Clarias batrachus* and *Channa punctatus* (120-130 gm) of 18-20 cm(standard length) were taken separately and kept in twenty groups and each group consist of forty eight fish species . No food was given to the above fish species during this period (08, 16 & 24hrs). The first set of *Labeo rohita* , *Clarias batrachus* and *Channa punctatus* were exposed to sub-lethal and lethal concentration of copper and zinc the detail were described somewhere else *[Shaffi & Kakaria,2006]*.

Preparation of tissue extract: The termination of the experiment preparation of tissue extract and enzyme assays were described elsewhere *[Colowick & Kaplon,1975 , Shaffi & Habbibulla,1977]*.

Statistical analysis: The experiments with acute and chronic studies were repeated at least seven times separately to subject the data for analysis of variance.

Table No.-1 : Combined iInfluence of *Chlorella vulgaris* & *Spirulina platensis* on Copper metal (Sub-lethal cons.) caused *hexokinase* variation in different brain regions of three freshwater teleosts. Acute studies

Regions of the Brain	Control	Duration of sub-lethal Concentration exposure			% of fall/ Rise	Duration of sub-lethal concentration exposure with *Chlorella vulgaris & Spirulina platensis*			% of fall/rise
		08 Hrs.	16 Hrs.	24 Hrs.		08 Hrs.	16 Hrs.	24 Hrs.	
		(A) *Labeo rohita (HAM)*							
Cerebrum	0.366 ±.052	0.222 ±.084	0.166 ±.032	0.117 ±.022	68.03	0.242 ±.032	0.189 ±.026	0.160 ±.024	56.28
Diencephalon	0.298 ±.036	0.162 ±.032	0.132 ±.024	0.084 ±.012	71.81	0.189 ±.024	0.155 ±.029	0.119 ±.014	60.06
Cerebellum	0.226 ±.030	0.144 ±.028	0.126 ±.018	0.113 ±.011	50.00	0.198 ±.022	0.172 ±.024	0.126 ±.019	44.24
Medulla Oblongata	0.335 ±.028	0.212 ±.022	0.174 ±.024	0.137 ±.022	59.10	0.295 ±.031	0.192 ±.022	0.170 ±.022	49.25
		(B) *Clarias batrachus*(LINN.)							
Cerebrum	0.351 ±.036	0.346 ±.036	0.196 ±.026	0.133 ±.018	62.10	0.334 ±.041	0.205 ±.019	0.178 ±.026	49.28
Diencephalon	0.254 ±.052	0.218 ±.022	0.148 ±.022	0.081 ±.012	68.11	0.242 ±.024	0.162 ±.021	0.124 ±.016	51.18
Cerebellum	0.171 ±.030	0.146 ±.019	0.124 ±.024	0.090 ±.016	47.36	0.162 ±.029	0.136 ±.016	0.102 ±.014	40.35
Medulla Oblongata	0.288 ±.028	0.177 ±.024	0.152 ±.019	0.129 ±.029	55.20	0.298 ±.032	0.162 ±.021	0.158 ±.021	45.13
		(C) *Channa punctatus* (BLOCH)							
Cerebrum	0.294 ±.042	0.264 ±.036	0.182 ±.036	0.129 ±.019	56.12	0.272 ±.024	0.192 ±.016	0.170 ±.026	42.17
Diencephalon	0.206 ±.018	0.189 ±.028	0.135 ±.022	0.074 ±.009	64.07	0.182 ±.018	0.126 ±.017	0.097 ±.018	52.91
Cerebellum	0.198 ±.034	0.182 ±.024	0.146 ±.019	0.114 ±.021	42.42	0.175 ±.016	0.141 ±.014	0.126 ±.022	36.36
Medulla Oblongata	0.254 ±.036	0.212 ±.020	0.168 ±.024	0.128 ±.016	49.60	0.224 ±.028	0.182 ±.022	0.154 ±.019	39.37

The data was subjected to test of ANOVA . Values are mean ± SDM of seven replicates . The super scripts a,b & c indicates that P >0.01, P>0.02, & P>0.05 respectively

Table No.-2 : Combined influence of *Chlorella vulgaris* & Spirulina platensis on copper metal (Lethal cons.) caused *hexokinase* variation in different brain regions of three freshwater teleosts. Acute studies

Regions of the Brain	Control	Duration of sub-lethal Concentration exposure			% of fall/ Rise	Duration of sub-lethal concentration exposure with *Chlorella vulgaris & Spirulina platensis*			% of fall/rise
		08 Hrs.	16 Hrs.	24 Hrs.		08 Hrs.	16 Hrs.	24 Hrs.	
		(A) *Labeo rohita (HAM)*							
Cerebrum	0.335 ±.126	0.246 ±.038	0.176 ±.029	0.083 ±.012	75.22	0.185 ±.026	0.152 ±.019	0.120 ±.018	64.17
Diencephalon	0.274 ±.082	0.189 ±.042	0.114 ±.034	0.052 ±.010	81.02	0.162 ±.018	0.134 ±.018	0.090 ±.015	67.15
Cerebellum	0.218 ±.046	0.172 ±.032	0.148 ±.028	0.089 ±.021	59.17	0.195 ±.029	0.138 ±.026	0.106 ±.016	51.37
Medulla Oblongata	0.320 ±.052	0.208 ±.026	0.166 ±.042	0.112 ±.018	65.00	0.296 ±.036	0.182 ±.019	0.147 ±.024	54.06
		(B) *Clarias batrachus*(LINN.)							

Cerebrum	0.318 ±.062	0.336 ±.024	0.194 ±.022	0.095 ±.015	70.12	0.296 ±.042	0.162 ±.024	0.120 ±.017	62.26
Diencephalon	0.241 ±.052	0.224 ±.030	0.132 ±.018	0.062 ±.010	74.27	0.218 ±.024	0.136 ±.018	0.089 ±.013	63.07
Cerebellum	0.174 ±.044	0.146 ±.028	0.122 ±.019	0.072 ±.012	56.09	0.151 ±.019	0.121 ±.017	0.086 ±.012	47.56
Medulla Oblongata	0.262 ±.040	0.156 ±.036	0.182 ±.034	0.107 ±.018	59.16	0.232 ±.042	0.148 ±.021	0.125 ±.021	52.29
		(C) *Channa punctatus* (BLOCH)							
Cerebrum	0.291 ±.036	0.254 ±.038	0.159 ±.024	0.108 ±.022	62.88	0.164 ±.028	0.142 ±.019	0.125 ±.036	56.01
Diencephalon	0.232 ±.024	0.176 ±.028	0.110 ±.022	0.069 ±.009	70.25	0.152 ±.036	0.122 ±.020	0.097 ±.017	58.18
Cerebellum	0.148 ±.032	0.182 ±.032	0.124 ±.032	0.077 ±.014	47.97	0.132 ±.022	0.121 ±.018	0.085 ±.012	42.56
Medulla Oblongata	0.242 ±.029	0.204 ±.068	0.148 ±.042	0.109 ±.019	54.95	0.212 ±.031	0.152 ±.014	0.135 ±.019	44.21

The data was subjected to test of ANOVA . Values are mean ± SDM of seven replicates . The super scripts a,b & c indicates that P >0.01, P>0.02, & P>0.05 respectively

Table No.-3 : Combined iInfluence of *Chlorella vulgaris* & *Spirulina platensis* on zinc metal (sub-lethal cons.) caused *hexokinase* variation in different brain regions of three freshwater teleosts. Acute studies

Regions of the Brain	Control	Duration of sub-lethal Concentration exposure			% of fall/ Rise	Duration of sub-lethal concentration exposure with *Chlorella vulgaris & Spirulina platensis*			% of fall/rise
		08 Hrs.	16 Hrs.	24 Hrs.		08 Hrs.	16 Hrs.	24 Hrs.	
		(A) *Labeo rohita (HAM)*							
Cerebrum	0.366 ±.098	0.198 ±.029	0.172 ±.032	0.146 ±.021	60.10	0.242 ±.036	0.195 ±.021	0.172 ±.028	53.00
Diencephalon	0.298 ±.064	0.178 ±.032	0.142 ±.024	0.099 ±.015	66.67	0.198 ±.023	0.164 ±.026	0.137 ±.019	54.02
Cerebellum	0.226 ±.048	0.159 ±.022	0.138 ±.019	0.120 ±.018	46.90	0.192 ±.019	0.148 ±.019	0.135 ±.013	40.26
Medulla Oblongata	0.335 ±.054	0.199 ±.032	0.162 ±.024	0.154 ±.019	54.02	0.305 ±.036	0.218 ±.022	0.184 ±.022	45.07
		(B) *Clarias batrachus*(LINN.)							
Cerebrum	0.351 ±.039	0.321 ±.066	0.236 ±.024	0.154 ±.021	56.12	0.318 ±.028	0.224 ±.016	0.207 ±.032	41.02
Diencephalon	0.254 ±.041	0.219 ±.022	0.148 ±.020	0.099 ±.018	61.02	0.232 ±.020	0.182 ±.019	0.144 ±.016	43.30
Cerebellum	0.171 ±.029	0.158 ±.019	0.124 ±.019	0.097 ±.016	43.27	0.151 ±.016	0.119 ±.021	0.106 ±.012	38.01
Medulla Oblongata	0.288 ±.039	0.266 ±.028	0.189 ±.014	0.149 ±.021	48.26	0.262 ±.032	0.195 ±.012	0.172 ±.024	40.27
		(C) *Channa punctatus* (BLOCH)							
Cerebrum	0.294 ±.041	0.266 ±.042	0.184 ±.026	0.147 ±.023	50.00	0.274 ±.029	0.196 ±.026	0.176 ±.018	40.13
Diencephalon	0.206 ±.028	0.178 ±.026	0.126 ±.018	0.091 ±.010	55.82	0.188 ±.019	0.132 ±.018	0.119 ±.021	42.23
Cerebellum	0.198 ±.036	0.162 ±.022	0.138 ±.014	0.120 ±.014	39.39	0.182 ±.022	0.142 ±.021	0.132 ±.014	33.53
Medulla Oblongata	0.254 ±.042	0.229 ±.019	0.168 ±.022	0.142 ±.019	44.09	0.236 ±.019	0.189 ±.021	0.165 ±.019	35.03

The data was subjected to test of ANOVA . Values are mean ± SDM of seven replicates . The super scripts a,b & c indicates that P >0.01, P>0.02, & P>0.05 respectively.

Table No.-4 : studiesCombined influence of *Chlorella vulgaris* & Spirulina platensis on zinc metal (lethal cons.) caused *hexokinase* variation in different brain regions of three freshwater teleosts. Acute studies

Regions of the Brain	Control	Duration of sub-lethal Concentration exposure			% of fall/ Rise	Duration of sub-lethal concentration exposure with *Chlorella vulgaris & Spirulina platensis*			% of fall/rise
		08 Hrs.	16 Hrs.	24 Hrs.		08 Hrs.	16 Hrs.	24 Hrs.	
		(A) *Labeo rohita (HAM)*							
Cerebrum	0.366 ±.098	0.210 ±.036	0.172 ±.022	0.113 ±.019	69.12	0.196 ±.026	0.182 ±.019	0.153 ±.024	58.19

Diencephalon	0.298 ±.064	0.184 ±.021	0.148 ±.019	0.081 ±.016	72.81	0.176 ±.014	0.142 ±.016	0.119 ±.018	60.06
Cerebellum	0.226 ±.048	0.154 ±.018	0.134 ±.021	0.101 ±.014	55.30	0.201 ±.018	0.182 ±.022	0.122 ±.021	46.01
Medulla Oblongata	0.335 ±.054	0.196 ±.022	0.158 ±.024	0.137 ±.016	59.01	0.307 ±.032	0.201 ±.023	0.170 ±.016	49.25
		(B) *Clarias batrachus*(LINN.)							
Cerebrum	0.351 ±.039	0.341 ±.032	0.189 ±.038	0.119 ±.017	66.09	0.319 ±.028	0.184 ±.018	0.161 ±.021	54.13
Diencephalon	0.254 ±.041	0.226 ±.028	0.136 ±.024	0.080 ±.012	68.50	0.224 ±.028	0.134 ±.018	0.114 ±.012	55.11
Cerebellum	0.171 ±.029	0.159 ±.019	0.118 ±.032	0.082 ±.014	52.04	0.156 ±.019	0.126 ±.016	0.097 ±.011	43.27
Medulla Oblongata	0.288 ±.039	0.242 ±.024	0.176 ±.012	0.129 ±.021	55.20	0.262 ±.026	0.184 ±.022	0.152 ±.016	47.22
		(C) *Channa punctatus* (BLOCH)							
Cerebrum	0.294 ±.041	0.178 ±.021	0.158 ±.022	0.126 ±.019	57.14	0.276 ±.032	0.181 ±.019	0.149 ±.021	49.31
Diencephalon	0.206 ±.028	0.184 ±.019	0.124 ±.018	0.080 ±.012	61.16	0.185 ±.024	0.142 ±.016	0.110 ±.014	46.60
Cerebellum	0.198 ±.036	0.169 ±.022	0.132 ±.026	0.112 ±.009	43.43	0.182 ±.019	0.132 ±.014	0.124 ±.016	37.37
Medulla Oblongata	0.254 ±.042	0.184 ±.032	0.149 ±.021	0.127 ±.022	50.00	0.236 ±.024	0.162 ±.024	0.149 ±.021	41.33

The data was subjected to test of ANOVA . Values are mean ± SDM of seven replicates . The super scripts a,b & c indicates that P >0.01, P>0.02, & P>0.05 respectively.

III. RESULTS

The combined impact of *Chlorella vulgaris* and *Spirulina platensis* was investigated on sub - lethal & lethal concentrations of copper and zinc toxicity on *hexokinase* in various brain regions of *Labeo rohita*(sub-lethal concentration of Zn- 0.72 mg/ltr. ,Cu- 0.10 mg/ltr and lethal concentration of Zn- 0.90 mg/ltr., Cu- 0.22 mg/ltr.), *Clarias batrachus* (sub-lethal concentration of Zn- 2.75mg/ltr. ,Cu- 0.50 mg/ltr and lethal concentration of Zn- 2.84 mg/ltr., Cu- 0.96 mg/ltr.), and *Channa punctatus* (sub-lethal concentration of Zn- 2.90mg/ltr. ,Cu- 0.80mg/ltr and lethal concentration of Zn- 3.08mg/ltr., Cu- mg/ltr.), at 08, 16 & 24 hrs. exposure under acute studies **.** Please see **Table-1 -4**.

The *hexokinase* fall was highest in diencephalon exposed to sub-lethal concentrations of copper in microbe presence (Two) at 08 hrs. than at 16 & 24 hrs. in comparison to cerebrum, medulla oblongata & cerebellum in *Labeo rohita* than in *Clarias batrachus* & *Channa punctatus***(Table-1-2)**.

The sub-lethal zinc exposed (In presence of two microbes) *Labeo rohita* registered highest fall in *hexokinse* activity in diencephalons in comparison to cerebrum, medulla oblongata & cerebellum at 08 hrs. than at 16 & 24 hrs. exposure than that of *Clarias batrachus* & *Channa punctatus* **(Table-3-4)**.

In all these investigations the fall in the above mentioned enzymes were optimum with copper & zinc exposed once respectively both at sub-lethal & lethal levels than in presence of microbes.

IV. DISCUSSION AND CONCLUSION

Heavy metal exposure causes enzyme inactivation, reduction in R.B.C., lifespan, fall in hemoglobin surface area, alteration in electron transport, damage to genetic material, immunological variations and change in bio-chemical makeup of different fish species [Bashiru & Rosemary, 2007; Murali & Mehar, 2014 & Nichat, 2018].

The Spirulina platensis influenced the sub-lethal & lethal effect of copper & zinc caused variations in brain compartmentation (cerebrum, diencephalons, cerebellum & medulla oblongata) of hexokinase in Labeo rohita, Clarias batrachus & Channa punctatus under acute or short term exposure. The sub-lethal and lethal levels of copper & zinc inhibited the hexokinase to a highest extent in diencephalon than in cerebrum, medulla oblongata & cerebellum in Labeo rohita in comparison to Clarias batrachus & Channa punctatus but lesser than the fall of the enzymes in the above said fish species directly exposed to sub-lethal & lethal levels of copper & zinc directly without any microbe compelled us to develop an insight to understand the positive impact on important bio-chemical parameters like enzymes that are important to promote a variety of anabolic & catabolic processes in an organism effectively reflects that microbes act as antidote effect fall heavy metal toxicity and the less fall of the four enzymes under investigation may be that microbes has a soothing impact and hence the microbes are able to decrease the sub-lethal & lethal toxicity of sub-lethal & lethal levels of copper & zinc.[Nichat et. al. 2014& Nichat, 2016 a]

The following finding may help to understand the microbe-metal interaction and sub sequent detoxification of the metal to a less extent in a better way *[Lu et al. ,2006; Kumar & Kalonia, 2007& Medhi et. al., 2008]* . The sub-cellular regions of Cyanobacteria and *Anabaena cylindrica* could trap the lead through its phosphate and precipitates in the form of lead phosphate on the cell wall inside the cell *[Sharma & Sharma, 2005*; *Manjrekar et. al., 2008; Ansari & Bhandari, 2008; Kaur & Bansal, 2008; Manousaki & Kalogerakis, 2009 Mench et. al., 2009 ; Bert et. al., 2009; Nichat, 2014]*. Similar kind of mechanism might have taken place in the present findings i.e. less fall of enzymes in which the cellular components of *Spirulina platensis* might have precipitated the metal into compound with the help of its cellular components and the present findings i.e. less fall of enzymes in presence of a autotroph than the enzyme fall when directly exposed to copper & zinc sub-lethal & lethal levels should understand on similar lines. Enhanced polyphosphate bodies formation were ascribed to heavy metal toxicity exposed group of animals and perhaps these bodies were suggested as the site of metal absorption in aquatic autotrophs *[Shaffi, & Kakaria,2006 & Nichat, 2016 b]*.

The potential negative surface charge of the poly-phosphate in the polyphosphate bodies will assist to absorb metal. Increase in the exposure time of autotrophs to heavy metals further increase the number of polyphosphate bodies & also composed of other materials such as magnesium, sodium, potassium, iron & copper *[Masoodi et. al., 2007; Manousaki et. al., 2009 & Bert et. al., 2009]*. Such bodies not only function in polyphosphate storage and further functions as a detoxification process such a mechanism is not rule out even in the present investigation and the fall of *hexokinase* with the metal exposure directly on one side and metal exposure in presence of *Spirulina* in *Labeo rohita*, *Clarias batrachus & Channa punctatus* on both side educates that the presence of the aquatic autotroph significantly checked the fall off the enzymes in different brain regions of the above said fish species is quite innovative and need further investigation on a large scale for the application in the aquatic system and to check the menace of pollution*[Shaffi, & Kakaria,2006 & Nichat et.al.,2015]*.

This investigation further helps that aquatic autotrophs can be used to remove heavy metals from aquatic system over a wide range of pH. Such events might have taken place even in the present investigation and the less fall in *hexokinase* in different brain regions of *Labeo rohita, Clarias batrachus* & *Channa punctatus* might be ascribed to a less degree in microbe presence than direct exposure to heavy metals.

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