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Research Article

Spirulina platensis and *Chlorella vulgaris* Assisted Bioremediation of Heavy Metal Contaminated Aquatic Ecosystem

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ABSTRACT

Living organisms require trace amounts of some heavy metals including copper, lead, magnesium, vanadium, zinc etc. Human activities have influenced bio- chemical & geological cycles. Metal ions become toxic in nature when they are beyond tolerance limit. In aquatic ecosystem, fishes & microbes have close, intimate & un separated contact from the embryonic to adult stage. Bioremediation is therefore an eco-friendly and efficient method of reclaiming environments contaminated with heavy metals by making use of the inherent biological mechanisms of microorganisms and plants to eradicate hazardous contaminants. Microbes play a key role in controlling the speciation & cycling of metals in water. Bio-availability, toxicity & reactivity of metals is greatly influenced to have a better understanding of the major factors that link microbial activity to the bio-geo-chemistry of metals. Micro-organism & other natural products [plants & animals & there by- products] capable of cycling metals for bio-remediation of contaminated site without any side effect on environment. This investigation discusses the toxic effects of heavy metal pollution and the mechanisms used by microbes for environmental remediation. It also emphasized the importance of modern techniques and approaches in improving the ability of microbial enzymes to effectively degrade heavy metals at a faster rate, highlighting recent advances in microbial bioremediation for the removal of heavy metals from the environment.

Keywords: *Chlorella vulgaris, Spirulina platensis*, Copper, *Zinc phosphoglucomutase*, *hexokinase*, *phosphoglucoisomerase* and *phosphofructokinase*, *Labeo rohita* (Ham.), *Clarias batrachus* (Linn.), *Channa punctatus* (Bloch.)

INTRODUCTION

Among the pollutants heavy metals are regarded as one of the most serious pollutants are due to their environmental persistence and tendency to concentrate in aquatic organisms. Heavy metals are chemical elements with a specific gravity that is at least five times greater than specific gravity of water and the pollution of ecosystem by heavy metal is an important problem. Heavy metals constitute some of the most hazardous substances that can bio-accumulates [1-4].

Heavy metals further affect organisms directly by accumulating in their body or indirectly by transferring to the next tropic levels of the food chain [5-7]. The accumulation of heavy metals in the viscera, precipitation leads into chronic illnesses and cause significant damage to various organisms including induced stress, lipid per oxidation, protein denaturation, DNA damage, decreases organism's life span and productivity of the natural water body [8,9].

The physiological, cellular & molecular mechanisms too used to regulate & detoxify environmental heavy metal toxicity on a variety of organisms but a clear understanding about the mechanism is awaited and expect further studies to establish a clear understanding on the above matter & through food & water, heavy metals/pollutants invariably find a place in the organisms including humans [2,6].

Heavy metal induces oxidative damage in different organs by increasing per-oxidation of membrane chemistry and altering the antioxidant system of the cells/tissues [10,11]. Interaction of metal ions with the cell organelles cause injury to cellular components. Heavy metal intoxication further depletes glutathione & protein bound sulfa-hydryl groups resulting into the production of reactive oxygen species like hydrogen peroxides, superoxide ions & hydroxyl radicals. These reactive oxygen species induce elevated visceral per-oxidation [9,12,13].

Researchers are innovating novel methods to clean up the heavy metal polluted water bodies by replicate transitional physical

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& chemical methods of environmental cleanup through phyto-remediation [10,14,15].

Hence the need of the man is to innovate some alternative technologies &devices to protect the nature gifted consumables and to boost the yield from natural water bodies [8,12,16]. Based on the above information, the autotrophic microbes like used as detoxifying agent on few economically, nutritionally & culturally important fish species have been selected for the present study.

The aim of this investigation is to determine the safety, sub-lethal and lethal concentration of copper & zinc, their impact on the bio-chemical compartmentation of carbohydrate metabolism enzymes *phosphoglucomutase*, *hexokinase*, *phosphoglucoisomerase* and *phosphofructokinase* in different brain regions of three nutritionally & economically important fish species *i.e, Labeo rohita* (Ham.), *Clarias batrachus* (Linn.) and *Channa punctatus* (Bloch.) on a comparative basis.

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 [17]. 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 [14].

Preparation of tissue extract

The termination of the experiment preparation of tissue extract and enzyme assays were described elsewhere [18,19].

Statistical analysis

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

RESULTS

The results enlightened that the combined influence of both the microbes (*Chlorella vulgaris* & *Spirulina platensis*) heavily decreased the toxic influence of copper & zinc on carbohydrate enzymes (*phosphoglucomutase, hexokinase, phosphoglucoisomerase & Phosphofructokinase*) in brain regions (cerebrum, diencephalons, cerebellum & medulla oblongata) in *Labeo rohita* (sub-lethal concentration of Zn- 0.72 mg/ltr, Cu- 0.10 mg/ltr), *Clarias batrachus* (sub-lethal concentration of Zn- 2.75mg/ltr, Cu- 0.50 mg/ltr), and *Channa punctatus* (sub-lethal concentration of Zn- 2.90mg/ltr, Cu- 0.80mg/ltr) under chronic studies (Table 1-8).

The sub-lethal copper concentration (in presence of two microbes) inhibited the phosphoglucomutase to a significant extent at thirty days exposure than in cerebrum, medulla oblongata & cerebellum in comparison to 15- & 45-days exposure in *Labeo rohita* (Table 1). In *Clarias batrachus* the fall in phosphoglucomutase was maximum in diencephalons at 30-day exposure followed by cerebrum, medulla oblongata at 15 days exposure & cerebellum at 30 days exposure than at 45 days exposure (*Table 1*).

In *Channa punctatus* the fall in *phosphoglucomutase* was highest in diencephalons at 30 days of exposure than in cerebrum, medulla oblongata & cerebellum at 15 days of exposure than at 45 days of exposure under chronic studies (Table 1).

The combined influence of *Chlorella vulgaris* & *Spirulina platensis* was experimented on sub-lethal concentrations of copper toxicity in which *hexokinase* registered optimum fall in diencephalons at 30 days of exposure followed by cerebrum & medulla oblongata at 15 days of exposure & cerebellum at 30 days of exposure than at 45 days of exposure in *Labeo rohita* (Table 2). In *Clarias batrachus* the *hexokinase* fall was recorded in diencephalons to a great extent at 15 days of exposure than in cerebrum, medulla oblongata & cerebellum in comparison to 30- & 45-days exposure (Table 2).

The *hexokinase* maximum fall was at 30 days exposure in diencephalons followed by cerebrum, medulla oblongata and cerebellum at 15 days of exposure than 45 days exposure under chronic studies in *Channa punctatus* (Table 2).

The *phosphoglucoisomerase* fall was optimum in diencephalon accompanied by cerebrum, medulla oblongata & cerebellum at 15 days exposure in *Labeo rohita* (Table 3) exposed to sub-lethal concentrations of copper in microbe's presence. In *Clarias batrachus* (Table 3) the *phosphoglucoisomerase* fall was highest in diencephalons at 30 days exposure to sub-lethal concentrations of copper in comparison to cerebrum, medulla oblongata & cerebellum at 15 days of exposure. The fall in *phosphoglucoisomerase* was noticed in diencephalons at 15 days of exposure accompanied by cerebrum, medulla oblongata & cerebellum under chronic studies in *Channa punctatus* (Table 3) than at 30 & 45 days of exposure.

The fall in *phosphofructokinase* was maximum in diencephalons at 30 days of exposure to sub-lethal concentrations of copper in presence of two microbes (*Chlorella vulgaris* & *Spirulina platensis*) in comparison to cerebrum, medulla oblongata (15 days exposure) & cerebellum (30 days exposure) in *Labeo rohita* (Table 4). In *Clarias batrachus* (Table 4) the fall in phosphofructokinase was noticed in diencephalons than in cerebrum, medulla oblongata & cerebellum at 15 days of exposure. The fall in *phosphofructokinase* was optimum at 30 days in dienecephalon accompanied by cerebrum, medulla oblongata and cerebellum at 15 days exposure to sub-lethal levels of copper in the microbe's presence in *Channa punctatus* (Table 4).

At 30 days exposure to sub-lethal concentrations of zinc the presence of two microbes affected *phosphoglucomutase* at 30 days in diencephalons than in cerebrum, medulla oblongata at 15 days & cerebellum in the *Labeo rohita* (30 days exposure) (Table 5). In *Clarias batrachus* (Table 5) the variations recorded in the phosphoglucomutase was prominent in diencephalons at 30 days followed by cerebrum, medulla oblongata (15 days exposure) and cerebellum (30 days exposure) than at 45 days exposure. The *phosphoglucomutase* fall was significant at 30 days in diencephalons

Table 1: Summarize of 16 Non-structural Proteins (NSP) in coronavirus and their function

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
		15 DAYS	**30 DAYS**	**45 DAYS**		**15 DAYS**	**30 DAYS**	**45 DAYS**	
(a) *Labeo rohita* (HAM)									
Cerebrum	0.421 ± .158	0.276 c ± .036	0.248 c ± .028	0.219 c ± .026	47.98	0.348 c ± .032	0.309 c ± .038	0.285 c ± .042	32.30
Diencephalon	0.242 ± .039	0.259 ± .042	0.162 c ± .019	0.126 c ± .024	57.85	0.248 ± .042	0.210 ± .024	0.192 ± .028	35.78
Cerebellum	0.229 ± .028	0.208 ± .022	0.169 ± .026	0.158 c ± .032	31.00	0.204 ± .028	0.198 ± .021	0.181 ± .016	20.96
Medulla Oblongata	0.336 ± .032	0.246 c ± .038	0.212 c ± .019	0.198 c ± .024	41.07	0.286 ± .036	0.258 ± .042	0.245 c ± .032	27.08
(b) *Clarias batrachus* (LINN.)									
Cerebrum	0.381 ± .081	0.268 c ± .028	0.236 c ± .020	0.228 c 0.036	40.15	0.314 ± .029	0.305 ± .019	0.297 ± .024	22.04
Diencephalon	0.268 ±. 042	0.252 ± .039	0.184 ± .021	0.136 c ± .016	49.25	0.212 ± .032	0.196 ± .021	0.187 ± .018	30.22
Cerebellum	0.188 ± .036	0.171 ± .021	0.149 ± .031	0.137 ±.019	27.12	0.170 ± .026	0.164 ± .028	0.154 ± .022	18.08
Medulla Oblongata	0.302 ± .028	0.236 ± .023	0.214 c ± .028	0.198 c ±.021	34.43	0.264 ± .038	0.252 ± .021	0.240 ± .032	20.52
(c) *Channa punctatus* (BLOCH)									
Cerebrum	0.301 ± .019	0.256 ± .042	0.224 ± .031	0.204 c ± .019	32.22	0.264 ± .042	0.256 ± .028	0.240 ± .032	20.26
Diencephalon	0.222 ± .022	0.198 ± .026	0.148 ± .024	0.133 c ± .022	40.09	0.194 ± .024	0.174 ± .019	0.166 ± .016	25.72
Cerebellum	0.161 ± .028	0.148 ± .022	0.134 ± .014	0.125 ± .019	22.36	0.140 ± .019	0.138 ± .012	0.135 ± .013	16.14
Medulla Oblongata	0.267 ± .032	0.247 ± .042	0.194 ± .021	0.187 ± .026	29.96	0.251 ± .022	0.234 ± .028	0.221 ± .021	17.22

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

Table 2: Combined influence of *Chlorella vulgaris & Spirulina platensis* on copper metal (sub-lethal) caused toxicity in three freshwater teleosts Hexokinase Chronic 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
		15 DAYS	**30 DAYS**	**45 DAYS**		**15 DAYS**	**30 DAYS**	**45 DAYS**	
(a) *Labeo rohita* (HAM)									
Cerebrum	0.342 ±.054	0.254 c ±.029	0.238 c ± .032	0.205 c ± .026	40.05	0.276 ± .041	0.254 c ± .032	0.239 c ± .041	30.11
Diencephalon	0.284 ±.038	0.254 ±.032	0.168 c ± .024	0.130 c ± .014	54.22	0.254 ± .028	0.199 c ± .019	0.181 c ± .021	36.26
Cerebellum	0.212 ±.029	0.198 ±.026	0.178 ± .012	0.154 ± .016	27.35	0.199 ± .032	0.186 ± .018	0.172 ± .026	18.86
Medulla Oblongata	0.314 ±.042	0.248 ±.032	0.222 c ± .019	0.197 c ±. 021	37.26	0.292 ± .038	0.256 ± .028	0.235 ± .032	25.15
(b) *Clarias batrachus* (LINN.)									
Cerebrum	0.321 ± .039	0.258 ± .028	0.232 c ± .024	0.228 c 0.019	28.97	0.292 ± .041	0.268 ± .031	0.256 ± .042	20.24
Diencephalon	0.232 ± .038	0.196 ± .026	0.146 c ± .018	0.125 c ± .014	46.12	0.289 ± .038	0.178 ± .022	0.169 ± .019	27.15
Cerebellum	0.158 ± .029	0.149 ± .019	0.134 ± .021	0.126 ± .011	20.25	0.146 ± .028	0.139 ± .016	0.132 ± .014	16.45

Medulla Oblongata	0.272 ± .042	0.238 ± .024	0.216 ± .019	0.204 ± .024	25.00	0.244 ± .039	0.232 ± .028	0.220 ± .026	19.11
(c) *Channa punctatus* (BLOCH)									
Cerebrum	0.280 ± .062	0.268 ± .028	0.218 ± .018	0.207 ± .021	26.07	0.244 ± .036	0.238 ± .018	0.229 ± .028	18.21
Diencephalon	0.196 ± .024	0.184 ± .024	0.136 ± .016	0.115 ± .009	41.32	0.182 ± .024	0.168 ± .019	0.150 ± .028	23.46
Cerebellum	0.182 ± .036	0.162 ± .019	0.158 ± .024	0.147 ± .016	19.23	0.169 ± .014	0.162 ± .021	0.156 ± .019	14.28
Medulla Oblongata	0.242 ± .040	0.221 ± .022	0.204 ± .019	0.191 ± .020	21.07	0.219 ± .022	0.210 ± .024	0.203 ± .024	16.11

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

Table 3: Combined influence of *Chlorella vulgaris & Spirulina platensis* on copper metal (sub-lethal) caused toxicity in three freshwater teleosts - Phosphoglucoisomerase - Chronic 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
		15 DAYS	30 DAYS	45 DAYS		15 DAYS	30 DAYS	45 DAYS	
(a) *Labeo rohita* (ham)									
Cerebrum	0.279 ± 042	0.216 ± .032	0.196 c ± .019	0.178 c ± .024	36.2	0.238 ± .046	0.219 ± .019	0.203 ± .022	27.24
Diencephalon	0.242 ± 036	0.186 ± .019	0.159 c ± .024	0.123 c ± .019	49.17	0.216 ± .028	0.184 ± .021	0.159 ± .018	34.29
Cerebellum	0.198 ± 024	0.184 ± .017	0.168 ± .018	0.152 ± .014	23.23	0.179 ± .019	0.171 ± .018	0.167 ± .021	15.65
Medulla Oblongata	0.261 ± 033	0.204 ± .024	0.192 ± .021	0.181 ± .022	31.03	0.221 ± .031	0.209 ± .022	0.201 ± .024	22.98
(b) *Clarias batrachus* (linn.)									
Cerebrum	0.272 ± 052	0.234 ± .032	0.218 ± .022	0.201 0.032	26.1	0.246 ± .032	0.236 ± .036	0.223 ± .032	18.01
Diencephalon	0.23 ± 022	0.178 ± .021	0.148 ± .018	0.133 ± .016	42.17	0.212 ± .022	0.186 ± .019	0.174 ± .016	24.34
Cerebellum	0.149 ± 019	0.134 ± .019	0.129 ± .016	0.123 ± .014	17.44	0.138 ± .019	0.132 ± .024	0.128 ± .014	14.09
Medulla Oblongata	0.24 ± 024	0.206 ± .028	0.192 ± .019	0.189 ± .021	21.25	0.226 ± .026	0.212 ± .019	0.199 ± .021	17.08
(c) *Channa punctatus* (bloch)									
Cerebrum	0.261 ± 019	0.231 ± .019	0.214 ± .021	0.198 ± .019	24.13	0.236 ± .019	0.222 ± .017	0.219 ± .022	16.09
Diencephalon	0.212 ± 018	0.184 ± .024	0.152 ± .014	0.133 c ± .014	37.26	0.182 ± .024	0.141 ± .010	0.167 ± .022	21.22
Cerebellum	0.129 ± 012	0.118 ± .019	0.112 ± .012	0.107 ± .016	17.05	0.12 ± .019	0.117 ± .014	0.113 ± .012	12.4
Medulla Oblongata	0.221 ± 021	0.196 ± .028	0.188 ± .024	0.174 ± .018	19	0.199 ± .022	0.19 ± .021	0.187 ± .016	15.38

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

in comparison to cerebrum, medulla oblongata (15 days exposure) & cerebellum under long term studies in *Channa punctatus* (Table 5).

The maximum fall in *hexokinase* in the presence of two microbes exposed to sub-lethal concentrations of zinc was in diencephalons at 30 days than cerebrum, medulla oblongata & cerebellum (15 days exposure) in *Labeo rohita* (Table 6) in comparison at 45 days of exposure. In *Clarias batrachus* (Table 6) the fall in *hexokinase* was noticed in diencephalons at 30 days prominently in comparison to cerebrum, medulla oblongata & cerebellum than at 15- & 45-days exposure. In *Channa punctatus* (Table 6) to the fall in *hexokinase* was significant in diencephalons at 30 days exposure accompanied by

Table 4: Combined influence of *Chlorella vulgaris & Spirulina platensis* on copper metal (sub-lethal) caused toxicity in three freshwater teleosts - Phosphofructokinase - Chronic 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
		15 DAYS	30 DAYS	45 DAYS		15 DAYS	30 DAYS	45 DAYS	
(a) *Labeo rohita* (ham)									
Cerebrum	0.258 ± 064	0.208 ± .021	0.184 ± .022	0.175 c ± .028	32.17	0.228 ± .032	0.208 ± .036	0.198 ± .026	23.25
Diencephalon	0.198 ± 026	0.182 ± .016	0.128 ± .014	0.110 c ± .012	44.44	0.182 ± .024	0.149 ± .019	0.138 ± .024	30.3
Cerebellum	0.149 ± 019	0.129 ± .014	0.122 ± .018	0.117 ± .014	21.47	0.141 ± .019	0.134 ± .018	0.129 ± .019	13.42
Medulla Oblongata	0.211 ± 022	0.172 ± .021	0.168 ± .024	0.152 ± .019	27.96	0.182 ± .024	0.178 ± .021	0.166 ± .018	21.32
(b) *Clarias batrachus* (linn.)									
Cerebrum	0.248 ± 032	0.212 ± .017	0.199 ± .032	0.194 0.034	21.77	0.228 ± .032	0.216 ± .024	0.208 ± .022	16.12
Diencephalon	0.168 ± 018	0.128 ± .019	0.116 ± .014	0.105 ± .016	37.5	0.149 ± .019	0.138 ± .019	0.131 ± .016	22.02
Cerebellum	0.13 ± 012	0.121 ± .014	0.116 ± .021	0.109 ± .014	16.15	0.119 ± .021	0.116 ± .019	0.114 ± .014	12.3
Medulla Oblongata	0.198 ± 019	0.179 ± .016	0.168 ± .024	0.164 ± .019	17.17	0.185 ± .035	0.172 ± .024	0.169 ± .022	14.64
(c) *Channa punctatus* (bloch)									
Cerebrum	0.235 ± 022	0.218 ± .021	0.198 ± .036	0.19 ± .021	19.14	0.226 ± .019	0.215 ± .022	0.202 ± .014	14.04
Diencephalon	0.138 ± 014	0.122 ± .017	0.102 ± .024	0.091 ± .014	34.05	0.124 ± .022	0.116 ± .016	0.111 ± .021	19.56
Cerebellum	0.109 ± 019	0.101 ± .019	0.099 ± .018	0.093 ± .012	14.67	0.102 ± .016	0.099 ± .018	0.097 ± .016	11
Medulla Oblongata	0.171 ± 018	0.156 ± .021	0.149 ± .024	0.143 ± .019	16.37	0.162 ± .018	0.155 ± .021	0.15 ± .018	12.28

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

Table 5: Combined influence of *Chlorella vulgaris & Spirulina platensis* on zinc metal (sub-lethal) caused toxicity in three freshwater teleosts - Phosphoglocomutase - Chronic 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
		15 DAYS	30 DAYS	45 DAYS		15 DAYS	30 DAYS	45 DAYS	
(a) *Labeo rohita* (ham)									
Cerebrum	0.419 ± 098	0.356 ± .064	0.324 c ± .048	0.301 c ± .026	28.16	0.364 ± .042	0.349 ± .066	0.335 c ± .075	20.04
Diencephalon	0.297 ± 064	0.258 ± .029	0.198 c ± .054	0.179 c ± .032	39.73	0.282 ± .036	0.232 ± .042	0.219 ± .019	26.26
Cerebellum	0.228 ± 038	0.206 ± .019	0.192 ± .029	0.184 ± .036	19.29	0.221 ± .021	0.209 ± .028	0.2 ± .024	12.28
Medulla Oblongata	0.332 ± 045	0.284 ± .032	0.268 ± .056	0.251 c ± .042	24.39	0.298 ± .036	0.288 ± .039	0.272 ± .029	18.07
(b) *Clarias batrachus* (linn.)									
Cerebrum	0.379 ± 056	0.343 ± .036	0.324 ± .044	0.303 c ± .019	20.05	0.343 ± .041	0.336 ± .039	0.326 ± .028	13.98

Diencephalon	0.266 ± 062	0.199 ± .024	0.184 ± .032	0.175 ± .021	34.21	0.242 ± .038	0.228 ± .024	0.212 ± .019	20.3
Cerebellum	0.186 ± 042	0.171 ± .025	0.164 ± .024	0.158 ± .019	15.05	0.179 ± .024	0.172 ± .019	0.165 ± .018	11.29
Medulla Oblongata	0.3 ± 039	0.278 ± .034	0.269 ± .039	0.252 ± .08	16	0.279 ± .029	0.269 ± .032	0.261 ± .022	13
(c) *Channa punctatus* (bloch)									
Cerebrum	0.299 ± 041	0.284 ± .036	0.264 ± .074	0.252 ± .019	15.71	0.282 ± .036	0.269 ± .041	0.261 ± .021	12.7
Diencephalon	0.22 ± 022	0.206 ± .019	0.169 ± .028	0.154 ± .014	30	0.209 ± .032	0.192 ± .028	0.184 ± .019	16.36
Cerebellum	0.159 ± 032	0.139 ± .022	0.137 ± .019	0.136 ± .016	14.46	0.149 ± .019	0.146 ± .024	0.143 ± .016	10.06
Medulla Oblongata	0.264 ± 042	0.239 ± .036	0.236 ± .024	0.224 ± .021	15.15	0.184 ± .021	0.162 ± .019	0.229 ± .014	12.87

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

Table 6: Combined influence of *Chlorella vulgaris & Spirulina platensis* on zinc metal (sub-lethal)caused toxicity in three freshwater teleosts - Hexokinase - Chronic 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
		15 DAYS	30 DAYS	45 DAYS		15 DAYS	30 DAYS	45 DAYS	
(a) *Labeo rohita* (ham)									
Cerebrum	0.34 ± 068	0.295 ± .032	0.272 ± .041	0.265 c ± .042	22.05	0.284 ± .036	0.264 ± .039	0.272 c ± .042	20
Diencephalon	0.282 ± 042	0.255 ± .028	0.198 c ± .022	0.183 c ± .016	35.1	0.262 ± .041	0.204 ± .024	0.189 c ± .032	32.97
Cerebellum	0.21 ± 036	0.196 ± .026	0.182 ± .036	0.174 ± .024	17.14	0.196 ± .028	0.184 ± .019	0.176 ± .024	16.19
Medulla Oblongata	0.31 ± 039	0.276 ± .041	0.259 ± .029	0.245 ± .021	26.96	0.264 ± .032	0.254 ± .024	0.241 ± .019	22.25
(b) *Clarias batrachus* (linn.)									
Cerebrum	0.319 ± 029	0.284 ± .039	0.272 ± .028	0.261 ± .019	18.18	0.284 ± .041	0.276 ± .036	0.269 ± .024	15.67
Diencephalon	0.23 ± 024	0.209 ± .028	0.184 ± .014	0.161 ± .017	30	0.212 ± .026	0.164 ± .019	0.239 ± .021	25.07
Cerebellum	0.156 ± 019	0.142 ± .019	0.139 ± .019	0.134 ± .015	14.1	0.132 ± .016	0.124 ± .024	0.139 ± .019	16.89
Medulla Oblongata	0.269 ± 021	0.246 ± .032	0.239 ± .022	0.228 ± .024	18.24	0.222 ± .019	0.164 ± .032	0.129 ± .018	17.3
(c) *Channa punctatus* (bloch)									
Cerebrum	0.278 ± 072	0.252 ± .031	0.248 ± .036	0.239 ± .021	14.02	0.254 ± .026	0.239 ± .032	0.244 ± .026	12.23
Diencephalon	0.194 ± 036	0.184 ± .032	0.159 ± .021	0.143 ± .014	26.28	0.178 ± .024	0.166 ± .024	0.157 ± .018	19.07
Cerebellum	0.18 ± 024	0.174 ± .020	0.164 ± .019	0.158 ± .016	12.22	0.172 ± .018	0.164 ± .019	0.158 ± .024	12.22
Medulla Oblongata	0.24 ± 036	0.222 ± .038	0.216 ± .022	0.206 ± .019	14.16	0.219 ± .019	0.212 ± .028	0.217 ± .018	9.58

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

Table 7: Combined influence of *Chlorella vulgaris & Spirulina platensis* on zinc metal (sub-lethal) caused toxicity in three freshwater teleosts - Phosphoglucoisomerase - Chronic 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
		15 DAYS	30 DAYS	45 DAYS		15 DAYS	30 DAYS	45 DAYS	
(a) *Labeo rohita* (ham)									
Cerebrum	0.276 ± 042	0.246 ± .032	0.238 ± .024	0.223 ± .029	19.2	0.242 ± .052	0.228 ± .024	0.23 ± .032	16.66
Diencephalon	0.24 ± 036	0.209 ± .028	0.182 ± .032	0.165 c ± .021	31.25	0.22 ± .026	0.188 ± .019	0.117 c ± .032	29.16
Cerebellum	0.196 ± 024	0.184 ± .026	0.172 ± .024	0.162 ± .032	17.34	0.182 ± .019	0.176 ± .022	0.168 ± .019	14.28
Medulla Oblongata	0.259 ± 032	0.238 ± .042	0.224 ± .018	0.212 ± .024	18.14	0.229 ± .032	0.212 ± .019	0.219 ± .021	15.44
(b) *Clarias batrachus* (linn.)									
Cerebrum	0.27 ± 042	0.246 ± .026	0.238 ± .026	0.226 ± .032	16.29	0.248 ± .036	0.236 ± .024	0.231 ± .022	14.14
Diencephalon	0.227 ± 028	0.196 ± .019	0.184 ± .019	0.165 ± .019	27.31	0.199 ± .026	0.189 ± .022	0.181 ± .014	20.26
Cerebellum	0.146 ± 019	0.139 ± .016	0.132 ± .024	0.128 ± .016	12.32	0.136 ± .024	0.131 ± .016	0.146 ± .015	9.58
Medulla Oblongata	0.237 ± 024	0.22 ± .032	0.214 ± .016	0.206 ± .026	13.08	0.216 ± .022	0.209 ± .032	0.209 ± .021	11.81
(c) *Channa punctatus* (bloch)									
Cerebrum	0.259 ± 041	0.239 ± .032	0.234 ± .022	0.228 ± .032	11.96	0.239 ± .041	0.229 ± .032	0.224 ± .024	9.65
Diencephalon	0.21 ± 036	0.184 ± .024	0.176 ± .032	0.161 ± .024	23.33	0.198 ± .028	0.184 ± .026	0.172 ± .019	18.09
Cerebellum	0.227 ± 019	0.119 ± .016	0.116 ± .016	0.114 ± .014	10.23	0.121 ± .019	0.116 ± .022	0.21 ± .021	7.48
Medulla Oblongata	0.219 ± 022	0.208 ± .019	0.199 ± .024	0.195 ± .025	10.95	0.202 ± .032	0.199 ± .024	0.2 ± .018	8.67

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

Table 8: Combined influence of *Chlorella vulgaris & Spirulina platensis* on zinc metal (sub-lethal) caused toxicity in three freshwater teleosts - Phosphofructokinase - Chronic 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
		15 DAYS	30 DAYS	45 DAYS		15 DAYS	30 DAYS	45 DAYS	
(a) *Labeo rohita* (ham)									
Cerebrum	0.258 ± 064	0.232 ± .042	0.224 ± .036	0.214 ± .024	17.05	0.229 ± .024	0.216 ± .032	0.216 ± .032	15.5
Diencephalon	0.198 ± 036	0.178 ± .026	0.166 ± .021	0.144 ± .018	27.27	0.178 ± .024	0.164 ± .019	0.151 ± .024	23.73
Cerebellum	0.149 ± 019	0.132 ± .018	0.129 ± .019	0.126 ± .016	15.43	0.141 ± .021	0.136 ± .014	0.131 ± .014	12.08
Medulla Oblongata	0.211 ± 022	0.198 ± .022	0.184 ± .016	0.177 ± .024	16.11	0.198 ± .026	0.184 ± .024	0.211 ± .018	13.27
(b) *Clarias batrachus* (linn.)									
Cerebrum	0.248 ± 036	0.222 ± .026	0.218 ± .019	0.213 ± .021	14.11	0.222 ± .032	0.216 ± .022	0.213 ± .022	12.9

Diencephalon	0.168 ± 014	0.152 ± .019	0.148 ± .016	0.132 ± .014	21.42	0.154 ± .016	0.128 ± .019	0.137 ± .018	18.45
Cerebellum	0.13 ± 012	0.126 ± .018	0.119 ± .016	0.117 ± .015	10	0.124 ± .019	0.119 ± .014	0.117 ± .016	9.06
Medulla Oblongata	0.198 ± 024	0.184 ± .014	0.176 ± .022	0.172 ± .018	13.13	0.182 ± .021	0.179 ± .024	0.175 ± .019	11.61
(c) *Channa punctatus* (bloch)									
Cerebrum	0.235 ± 042	0.226 ± .028	0.215 ± .024	0.209 ± .022	11.06	0.219 ± .024	0.209 ± .019	0.211 ± .026	10.21
Diencephalon	0.138 ± 026	0.12 ± .018	0.116 ± .014	0.113 ± .012	18.11	0.132 ± .016	0.119 ± .021	0.115 ± .016	16.66
Cerebellum	0.109 ± 019	0.105 ± .014	0.101 ± .010	0.099 ± .012	9.17	0.104 ± .012	0.102 ± .016	0.142 ± .012	7.33
Medulla Oblongata	0.171 ± 018	0.161 ± .021	0.159 ± .023	0.153 ± .026	10.52	0.162 ± .024	0.156 ± .019	0.157 ± .017	8.18

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

cerebrum, medulla oblongata (15 days exposure) and cerebellum (30 days exposure) under long term studies.

The fall in *phosphoglucoisomerase* was maximum at 30 days in diencephalons in comparison to cerebrum, medulla oblongata (15 days) & cerebellum (30 days) exposed to sub-lethal concentrations of zinc in the presence of two mibrobes in *Labeo rohita* (Table 7) than at 45 days of exposure. In *Clarias batrachus* (Table 7) the *phosphoglucoisomerase* fall was highest in the diencephalons at 30 days of exposure to zinc in the presence of microbes than 15- & 45-days of exposure than in cerebrum, medulla oblongata & cerebellum (15 days exposure). The sub-lethal concentrations of zinc manifested optimum enzyme variation in diencephalons at 30 days than at 15- & 45-days exposure accompanied by cerebrum, medulla oblongata (15 days exposure) and cerebellum (30 days exposure) under long term studies in *Channa punctatus* (Table 7).

The sub-lethal concentrations of zinc in presence of two microbes described earlier manipulated *phosphofructokinase* (Table 8) to a marked extent in diencephalons at 30 days exposure than in cerebrum, medulla oblongata (15 days exposure) and cerebellum (30 days exposure) in *Labeo rohita*. In *Clarias batrachus* (Table 8) also the diencephalon *phosphofructokinase* registered highest fall at 30 days exposure than in cerebrum, medulla oblongata (15 days) & cerebellum (30 days) exposure to sub-lethal level of zinc in presence of microbes.

The trend in phosphofructokinase fall exposure to sub-lethal concentrations of zinc in the presence of two microbes in *Channa punctatus* is more of less similar to *Labeo rohita* & *Clarias batrachus* under chronic studies (Table 8).

DISCUSSION AND CONCLUSION

The mechanism of detoxification of copper & zinc may be visualized as aquatic autotrophs has been used in industrial but also in domestic uses like water treatment as they are capable of removing waste to a great extent.

The uptake of copper & zinc by aquatic autotrophs used in present investigation has been realized that the aquatic autotrophs have an initial rapid stage and a slower stage. During rapid phase the metal ions are absorbed on the surface and transport them across the cell membrane & it is the first symptom of cell damage & deterioration of membranes [4,10,12,20].

It is further observed that there is an increase in the number of polyphosphate bodies with heavy metal toxicity in cyanobacteria as polyphosphate bodies have been working as indicators of metal absorption in cyanobacteria. The strong negative surface charge of polyphosphate in the phosphate bodies may help in absorbing the metal. The polyphosphate bodies may contain magnesium, sodium, iron & phosphorous. The polyphosphate bodies may not function in storage of polyphosphate but also help in detoxification mechanism [10,14,21,22].

The cyanobacteria further contain cyanophin granules that act as storage in the cell. Perhaps these bodies may participate in the cell internal detoxification process. The pH of the media may also influence the toxicity of copper & zinc by altering the form/nature of heavy metals [2,20].

Hydrogen ions may also play a vital role to check the toxic impact of copper & zinc as the metal binding sight on the cell surface binds with a proton reflects. Those protons will compete with metal ions for the binding sight. Change in gases ratio in aquatic system may change the temperature of the media by that aquatic autotrophs may absorb heavy metals [23-25].

The above-mentioned episodes are not totally/partially ruling out even in the present investigation and the fall in *phosphoglucomutase, hexokinase. phosphoglucoisomerase* & *phosphofructokinase* in cerebrum, diencephalons & medulla oblongata in *Labeo rohita, Clarias batrachus* & *Channa punctatus* with direct sub-lethal and lethal metal exposure and in presence of aquatic autotrophs & their cell organization bound mechanism of' detoxification to neutralize the sub-lethal & lethal copper & zinc concentrations affect the acute & chronic studies prominently reflect that without the possibilities of the above mentioned & discussed processes it was not possible for *Chlorella vulgaris* & *Spirulina platensis* to detoxify the metal caused toxicity on *phosphoglucomutase, hexokinase, phosphoglucoisomerase* & *phosphofructokinase* in *Labeo rohita, Clarias batrachus* & *Channa punctatus* on a comparative basis from a tropical habitat.

The finding may help to understand the microbe-metal interaction and sub sequent detoxification of the metal to a less extent in a better way. 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 [8,9,12,26-28].

The following mechanisms are used for microbial bioremediation:

(1) Sequestration of toxic metals by cell wall components or by intracellular metal binding proteins and peptides such as metallothioneins (MT) and phytochelatins along with compounds such as bacterial siderophores which are mostly catecholates, compared to fungi that produce hydroxamate siderophores.

(2) Alteration of biochemical pathways to block metal uptake.

(3) Conversion of metals to innocuous forms by enzymes.

(4) Reduction of intracellular concentration of metals using precise efflux systems.

The mechanisms used in remediation of heavy metals from contaminated soils are presented in following (Figure 1).

Mechanisms of removal of heavy metals from contaminated soils by microorganism through the processes of precipitation, biosorption via sequestration by intracellular metal binding proteins (metallothioneins) and conversion of metals to innocuous forms by enzymes (enzymatic transformation).

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 was ascribed to heavy metal toxicity exposed group of animals and perhaps these bodies were suggested as the site of metal absorption in aquatic autotrophs [13,20,27,29,30].

The physico-chemical factor of the water body is most affected due to continuous discharge or dumping toxicants from different source. Alteration in the physico-chemical parameters of the habitat would generate stress and this stress not only influences other organisms but their function including of the water. The pH of the water media certainly reduces the toxicity of cadmium & zinc to the fish in general and nervous system in particular and forms a compound with hydroxyl group such a mechanism may not be ruled out as *Spirulina platensis* has a higher absorption capacity for heavy metals and the fall in the above said enzymes is less in aquatic microbe presence than in direct metal exposure *i.e.*, the metal complex formation with hydroxyl group might be higher in the diencephalons in comparison to cerebrum, medulla oblongata & cerebellum in *Labeo rohita* than in *Clarias batrachus* & *Channa punctatus*.

The heavy metal removal significantly affected by the pH in the solution as hydrogen ions plays an important role in multicomponent absorption system. The increase in heavy metal uptake by autotroph *Spirulina platensis* & *Cynobacteria* with the increasing pH. A pH dependence of ion generally occurs when heavy metal binding site on cell surface binds with proton. This indicate that the protons will compete with metal ions for the binding site. Hence most ions are absorbed at a highest pH in a better way due to lower competition with protons. This indicates that heavy metals were smartly absorbed in a pH range of 4-8 [11,23,31,32].

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 [12,20,34-36]. 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 *phosphoglucomutase*, *hexokinase*, *phosphoglucoisomerase* and *phosphofructokinase* 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 [3,12,15,37].

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 *phosphoglucomutase*, *hexokinase*,

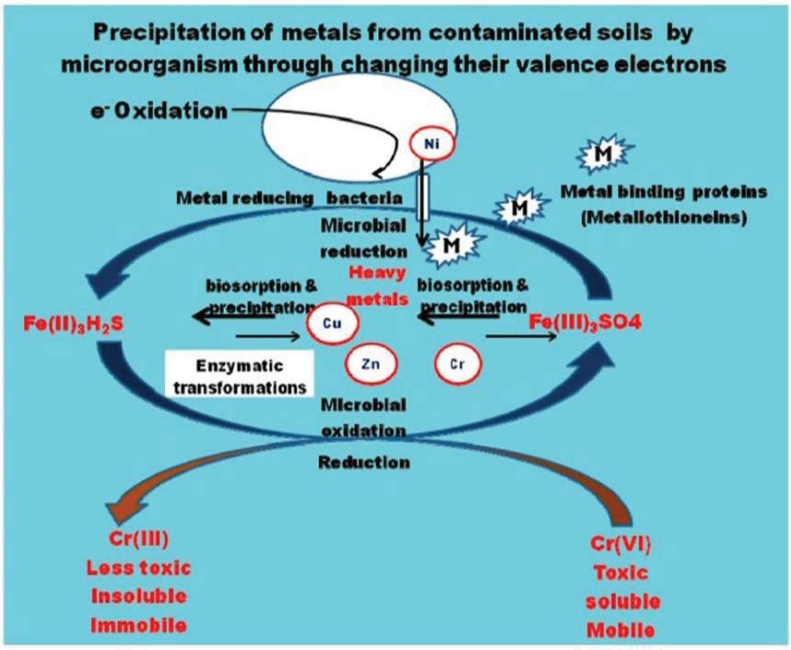

Figure 1: The mechanisms used in remediation of heavy metals from contaminated soils are represented.

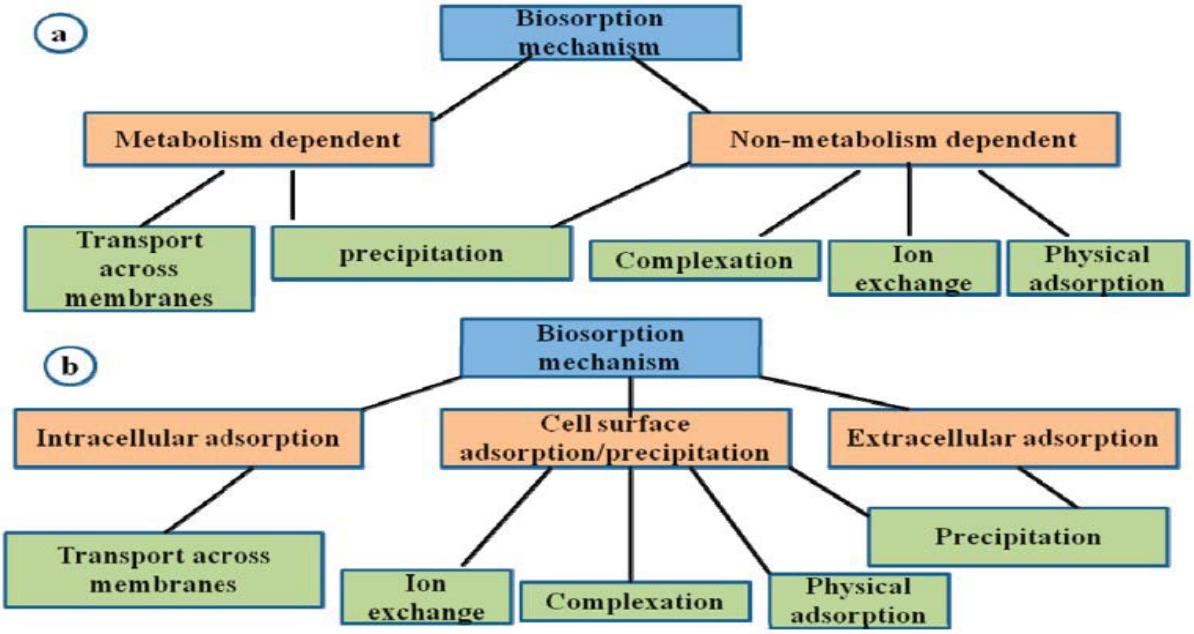

Figure 2: Mechanisms of biosorption based on (a) Dependence on cell metabolism; (b) Location within the cell where the metal is removed.

phosphoglucoisomerase & *phosphofructokinase* 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.

REFERENCES

1. Nematia MA, Kazemein F. Accumulation of Pb, Zn, C and Fe in plants and hyperaccumulator choice in galali iron mine area, Iran. Int J Agric Crop Sci. 2013;5:426-432.
2. Kang C, Kwon Y, So J. Bioremediation of heavy metals by using bacterial mixtures. Ecol Eng. 2016;89:64-69.
3. Ansari MN, Bhandari U. Protective effect of Emblica ribes Burm on methionine induced hyperhomocysteinemia and oxidative stress in rat brain. Ind J Exp Biol. 2008;46:521-527.
4. Manjrekar AP, Jisha V, Bag PP, Adhikary B, Pai MMH, Nandini M. Effect of Phyllanthus niruri (Linn.) treatment liver, kidney and testes in CCl4 induced hepatotoxic rats. Ind J Exp Biol. 2008;46:514-520.
5. Dixit R, Malaviya D, Pandiyan K, Singh UB, Sahu A, Shukla R, et al. Bioremediation of heavy metals from soil and aquatic environment: An overview of principles and criteria of fundamental processes. Sustainability. 2015;7:2189–2212.
6. Ndeddy Aka RJ, Babalola OO. Effect of bacterial inoculation of strains of pseudomonas aeruginosa, alcaligenes feacalis and Bacillus subtilis on germination, growth and heavy metal (Cd, Cr, and Ni) uptake of Brassica juncea. Int J Phytoremediation. 2016;18:200–209.
7. Tak HI, Ahmad F, Babalola OO. Advances in the application of plant growth-promoting rhizobacteria in phytoremediation of heavy metals. Rev Environ Contam Toxicol. 2013;223:33-52.
8. Masoodi MH, Khan SA, Shah MY, Khan S, Ahmed B. Hepatoprotective activity of Lychinis coronaria L. in carbon tetra chloride induced toxicity. J Pharmaceu. 2007;6:190-192.
9. Chandra K, Salman AS, Mohd A, Sweety R, Ali KN. Protection against face induced oxidative stress induced DNA damage as a model of arthritis and in vitro anti-arthritic potential of costus speciosus rhizome extract. Int J Pharm Phytopharmacol Res. 2015;7:383-389.
10. Ayangbenro AS, Babalola OO. (2017). A new strategy for heavy metal polluted environments: A review of microbial biosorbents. Int J Environ Res Public Health. 2017;7;14:94.
11. Kumar A, Kalonia H. Protective effect of Withania somnifera on the behavioral and biochemical alteration in sleep-disturbed mice (Gride over water suspended method). Ind J Exp Biol. 2007;45:524-528.
12. Fu YQ, Li S, Zhu HY, Jiang R, Yin LF. Biosorption of copper(II) from aqueous solution by mycelial pellets of rhizopus oryzae. Afr J Biotechnol. 2012;11:1403-1411.
13. Kaur J, Bansal MP. Effect of Vitamin-E on alcohol-induced changes in oxidative stress and expression of transcription factors NFKB and Ap-1 in mice brain cerebral hemispheres. Ind J Exp Biol. 2008;46:562-567.
14. Shaffi SA. Kakaria VK. Comparison of the sub-lethal effect of metal mixture on gluconeogenic enzymes compartmentation and recovery in brain of three fresh water teleosts. J Cell Tissue Res. 2006;6:3-17.
15. Bert V, Seuntjens P, Dejonghe W, Lacherez S, Thuy HT, Vandecasteele B. Phyto-remediation as a management option for contaminated sediments in tidal marshes, flood control areas and dredged sediment land fill sites. Environ Sci Poll Res. 2009;16 :745-64.
16. Verma JP, Jaiswal DK. Book review: Advances in biodegradation and bioremediation of industrial waste. Front Microbiol. 2016;6:pp1555.
17. Finney DT. Probit Analysis Method. 2nd edn. Camb Uni. Press, United States. 1971
18. Colowick SP. Kaplon, NO. Methods in Enzymology. Aca Press. New York. 1975;41:5.
19. Shaffi SA, Habibulla M. Differential distribution of glycogen, lactate & pyruvate indifferent brain regions of rat. Ind J Exp Biol. 1977;14:307-308.
20. Saquing JM, Yu YH, Chiu PC. Wood-derived black carbon (biochar) as a microbial electron donor and acceptor. Environ Sci Technol Lett. 2016;3:62-66.
21. Jan AT, Azam M, Ali A, Haq QMR. Prospects for exploiting bacteria for bioremediation of metal pollution. Crit Rev Environ Sci Technol. 2012;44:519-560.
22. Nichat AR, Kakariya VK, Shaffi SA. Influence of Chlorella vulgari on copper metal caused phosphoglucomutase variations in different brain regions of teleosts due to detoxification. J Industrial Poll Control. 2014;30.
23. Kanmani P, Aravind J, Preston D. Remediation of chromium contaminants using bacteria. Int J Environ Sci Technol. 2012;.9:183-193.

24. Manousaki E, Kalogerakis N. Phytoextraction of Pb and Cd by the Mediterranean saltbush (Atriplex halimus L.): Metal uptake in relation to salinity. Environ Sci Pollu Res. 2009;16: 844-854..

25. Medhi B, Prakash A, Avti PK, Saikia UN, Pandhi P, Khanduja KL. Effect of Mannka honey and sulfasalazine in combination to promote antioxidant defense system in experimentally induced ulcerative colitis model in rats. Ind J Exp Biol. 2008;46:583-590.

26. Nichat AR, Kakariya VK, Shaffi SA. Zinc metal caused hexokinase variations in different brain regions of teleosts and influence of Chlorella vulgaris with the special reference of detoxification. IOSR Journals. 2015;1:10-14.

27. Lee YC, Chang SP. The biosorption of heavy metals from aqueous solution by Spirogyra and Cladophora filamentous macroalgae. Bioresour Technol. 2011;102:5297-5304.

28. Ojuederie OB, Babalola OO. Microbial Assisted Bioremediation of Heavy Metal Polluted Aquatic Environments: A Review. Int J Environ Res Pub Health. 2017;14:1504.

29. Bashiru BO, Rosemary IE. Heavy metals (Lead, Cadmium, & Mercury) accumulation in the body tissue of Pachymelania aurita (Muller). Inter J Ecol Environ Sci. 2007;33:301-307.

30. Lu HK, Hsieh CC, Hsu JJ, Yang YK, Chou HN. Preventive effects of Spirulina platensis on skeletal muscle damage exercise –induced oxidative stress. Eur J Appl Physiol. 2006;98:220-226.

31. Bano M, Vyas R, Bist R, Bhatt DK. Protective role of combination of certain antioxidants against lindane (y-HCH) induced olfactory dysfunction in mice. J Cell & Tissue Res. 2007;7:26.

32. Murali O, Mehar SK. Bioremediation of heavy metals using Spirulina. Int J Geol Earth Environ Sci. 2014;4:244-249.

33. Gupta A, Joia J, Sood A, Sood R, Sidhu C, Kaur G. Microbes as potential tool for remediation of heavy metals: A review. J Microb Biochem Technol. 2016;8:364-372.

34. Mench M, Schwitzguébel JP, Schroeder P, Bert V, Gawronski S, Gupta S. Assessment of successful experiments and limitations of phytotechnologies: Contaminant uptake, detoxification and sequestration, and consequences for food safety. Environ Sci Pollu Res. 2009;16:876.

35. Nichat AR. Role of Spirulina platensis on zinc metal caused phosphoglucoisomerase variations in brain regions of fresh water fishes. IJISET. 2016;3:429-438.

36. Nichat AR. Combined impact of Chlorella vulgaris and Spirulina platensis on copper metal caused variations of phosphoglucomutase enzyme. IESRJ. 2016;2:21-23.

37. Aniko KP, Ferenc K, Attila F, Timea P. Biosorption characteristics of Spirulina & Chlorella cells for the accumulation of heavy metals. J Serb Chem Soc. 2015;80:407-419.