

## Effect of Toxicity on the *CYPRINUS CARPIO* fish

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### **Abstract**

The lotic aquatic environment has been polluted by effluents wastes having toxic trace metals from anthropogenic activities. A major portion of these toxic metals accumulates in the body of aquatic organisms, hence these organisms are often considered as potential indicators of aquatic metal pollution. These metals are harmful to animal itself and also to consumers in the food chain after a permissible concentration. *CYPRINUS CARPIO* of being end consumers in the aquatic food chain are used as the indicators of heavy metal enrichment. Much attention has been paid to biomonitoring of these trace metals after minimata diseases caused by mercury and cadmium contamination in the aquatic environment. Studies on the heavy metal contents in *CYPRINUS CARPIO* of various localities have been reported. The riverine *CYPRINUS CARPIO* of the district Patna despite the fact that the both rivers Ganga River highly sensitive for metals pollution and contribute more than half in total fish production of this district.

**Keywords:** *Pollutants, heavy metals, CYPRINUS CARPIO , River Ganga, Patna.*

### **Introduction**

No organic life can develop and survive without the participation of metal (element) ions. Basically the metals are divided into two categories; the macro-elements and micro-elements (trace elements or heavy metals). Essential macroelements to life are Sodium, Potassium, Magnesium, Calcium etc. Essential microelements to life are zinc, manganese, iron, cobalt, copper, molybdenum etc. All metals essential to life are toxic when supplied in concentrations in excess of the optimum concentration levels. But there is a group of trace metals which are nonessentials to life and has become synonymous with poison, such as arsenic, mercury, cadmium, lead etc.

The lotic aquatic environment has been polluted by effluents wastes having toxic trace metals from anthropogenic activities. A major portion of these toxic trace metals accumulates in the body of aquatic organism, hence these organisms are often considered as potential indicator of aquatic metal pollution. These metals are harmful to animal itself and also to consumers in food chain after a permissible concentration. CYPRINUS CARPIO are the end consumers in aquatic food chain and thus these are used as indicators of heavy metals enrichments. Biomonitoring of heavy metal pollution has been given ever-increasing attention due to ability of various fresh water organisms to accumulate trace elements<sup>1</sup>. Biological monitoring may be defined as the systematics or repetitive measurement and assessment of agents or their metabolites either in tissues or secretions to evaluate the uptake of a chemical and the health risk involved, with the purpose of prevention of health effects by corrective actions as needed<sup>2</sup>. Much attention has been paid to biomonitoring of these trace metals after minamta<sup>3</sup> and itai-itai<sup>4</sup> diseases caused by mercury and cadmium contamination in the aquatic environment.

Studies on the heavy metal contents in CYPRINUS CARPIO (whole fish/different organs) have been reported by a large number of workers such as Ruparelia et.al.<sup>5</sup>, Srivastava<sup>6</sup> Ayyadurai et.al.<sup>7</sup>, Pandey et.al.<sup>8</sup>. Different workers have been also reported on heavy metal contents in blood of different animals like Lizard<sup>9</sup>, Aves<sup>10</sup>, Beef steers<sup>11</sup> and Raccoons<sup>12</sup>.

Blood is the biological medium most commonly used in monitoring exposure to heavy metals because blood act as distributing medium to whole body and toxic metals reach to muscles and other organs via this medium and accumulate there.

District Patna has two prominent rivers the Sai (95km) and the Ganga (86 km) and both are highly sensitive for metal pollution<sup>13</sup>. However it appears from the literature that no attempt has been made to assess the concentration of trace metals in CYPRINUS CARPIO from both the rivers in this district despite the fact that both rivers contribute more than half in total fish production of this district<sup>14</sup>. Hence the present studies have been under taken to find out the concentration of seven trace metals in blood and muscle tissue of three edible CYPRINUS CARPIO of high food value of the rivers.

### **Material and Methods**

To study trace metal concentration in blood and muscle three edible CYPRINUS CARPIO were selected belonging to three chief fresh water CYPRINUS CARPIO groups; Labeorohita

from major carps Clariasbatrachus from cat CYPRINUS CARPIO and 3, Channa striatus from live CYPRINUS CARPIO . Whole blood samples were collected in the heparinized containers. Blood samples (min. 2ml. to max. 5ml.) were obtained by direct puncturing the heart with disposable syringe of 5ml. size. Fresh muscle tissue and blood samples were digested with 15ml. (each sample x 5ml. x 3 times) concentrated analytical grade nitric acid (Co. Ranbaxy) in conical flask on hot plate at a temperature of 70°C till the white or creamy or light pale residue appeared. The digested masses (Colourless residue) were diluted with 10ml. of distilled water. now the concentrations of cadmium (Cd) , copper (Cu), chromium (Cr), maganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) in digested masses were determined by atomic absorption spectrophotometer (Perkin/Elmer AAS model 5000) as mentioned in perkin elemer Mannual<sup>15</sup> , Bais et.al.<sup>16</sup>.

Blank sample were also prepared to check impurities in different chemical self. These blank samples were digested as above, and measured in atomic absorption spectrophotometer with true samples for each metals.

**Observations:** The level of concentration of trace metals cadmium, copper, chromium, mangnese, nickel, lead and zinc in blood and muscle tissue of three selected edible CYPRINUS CARPIO , Labeorohita, Channastriatus, and Clariasbatrachus of the river has been presented in table-1 and 2.

Table-1									
Concentration of toxic metals in the blood and muscles of CYPRINUS CARPIO of River at Patna									
Sampling Station	Pisces Species	Sample	Cd	Cu	Cr	Mn	Ni	Pb	Ni
Mahendrughat A	Blood		0.12±	BDL	0.50±	0.50 ±	0.35	0.15	6.45±
			0.04		0.12	0.08	±0.08	±0.04	0.38
	Muscle		0.21±	0.12±	1.32±	1.17±	1.14±	0.24±	6.36±
			0.06	0.04	0.26	0.21	0.21	0.04	0.54
	Blood		0.09±	0.06	0.26±	0.31±	0.28±	0.08	
			0.02	±0.02	0.06	0.07	0.10	±0.02	4.38±0.22

			0.17±	0.24	1.16±	0.89±	0.94±	0.22±	8.22±
		Muscle							0.43
			0.05	±0.06	0.22	0.14	0.18	0.06	
			0.14	0.08±	0.22	0.38±	0.27	0.11	4.68
	C	Blood							±0.33
			±0.03	0.03	±0.06	0.12	±0.07	±0.03	
			0.08±	0.42±	0.83±	0.73±	1.22±	0.31±	7.39±
		Muscle							0.41
			0.02	0.16	0.08	0.13	0.22	0.04	
Dighaghat	A	Blood	0.18±	0.10±	0.46±	0.86±	0.42±	0.19±0.0	9.88±
									0.28
			0.06	0.03	0.10	0.25	0.14	4	
			0.22±	0.31±	0.97±	1.74	1.62±	0.28±	1.21±
		Muscle							0.16
			0.04	0.06	0.27	±0.26	0.30	0.06	
			0.04±	0.06±	0.28±	0.41±	0.39		5.03±
	B	Blood						03 ±0.01	0.25
			0.01	0.01	0.08	0.08	±0.09		
			0.06±	0.29±	1.41	1.82±	1.88±	0.17±	9.17±
		Muscle							0.39
			0.02	0.09	±0.32	0.34	0.23	0.05	
			0.14	0.04		0.19	0.31±	0.17±	5.39
	C	Blood			0.27±0.06				±0.40
			±0.05	±0.01		±0.06	0.07	0.03	
			0.28±	0.22±	1.03±	0.43±	1.53±	0.34±	2.20±
		Muscle							0.29
			0.08	0.04	0.17	0.17	0.29	0.09	
Gaygath									
	A	Blood	Na	Na	Na	Na	Na	Na	Na

		Muscle	Na	Na	Na	Na	Na	Na	Na
			0.28±	0.14±	0.39±	0.88±	0.44±	0.21±	10.66±
	B	Blood							
			0.04	0.05	0.11	0.14	0.12	0.09	0.48
			0.88±	1.02±	2.24±	2.49±	2.24±	1.04±	13.32±
		Muscle							
			0.14	0.21	0.39	0.30	0.36	0.11	0.66
			0.24±	0.17±	0.48±	0.67±	0.38±	0.16±	12.21
	C	Blood							
			0.07	0.04	0.13	0.16	0.08	0.04	±0.34
			0.76±	1.06±	2.86±	2.67	2.14	0.83±	14.66±
		Muscle							
			0.13	0.18	0.28	±0.37	±0.27	0.19	0.55
All the values are mean ± S.E. of 3 observation. Pisces species:						blood are in ppm,			
A=Labeorohita, Values in						B=Channa striatus			
Values in muscles tissue are in			C=Clarias batrachus Na = Sample not available., BDL=						
µg/gm.,			below detectable limit.						

**Cadmium (Cd):** In river Sai CYPRINUS CARPIO the range of cadmium was recorded table-1 in between  $0.04 \pm 0.01$  ppm and  $0.28 \pm 0.04$  ppm in blood while it was  $0.06 \pm 0.02$  µg/g to  $0.88 \pm 0.14$  µg/g in muscles. Highest concentration ( $0.88 \pm 0.14$  µg/g) was observed in muscle tissue of carnivorous fish C. striatus.

In gangetic CYPRINUS CARPIO the concentration of cadmium table-2 was in range of  $0.12 \pm 0.03$  ppm to  $0.42 \pm 0.10$  ppm in blood and  $0.53 \pm 0.13$  µg/g to  $1.42 \pm 0.23$  µg/g in muscles. Concentration of Cd was highest ( $1.42 \pm 0.23$  µg/g) in muscles of carnivorous fish C. striatus.

**Copper(Cu):** The range of copper concentration in blood and muscle tissue of river Table-1 CYPRINUS CARPIO was from , BDL to 0.17

$0.04$  ppm and  $0.12 \pm 0.04$  µg/g to  $1.06 \pm 0.18$  µg/g respectively. Omnivorous fish Clarias batrachus show highest concentration of Cu in muscle tissue ( $1.06 \pm 0.18$  µg/g).

In Gangetic CYPRINUS CARPIO copper ranged in between  $0.02 \pm 0.01$  ppm to  $0.14 \pm 0.05$  ppm in blood and  $9.53 \pm 0.31$  µg/g to  $31.62 \pm 3.24$  µg/g in muscles. Concentration of Cu was highest in muscle of carnivorous fish C. striatus ( $31.62 \pm 3.24$  µg/g).

**Chromium (Cr):** The range of this trace metal was recorded from  $0.22 \pm 0.06$  ppm to  $0.50 \pm 0.12$  ppm in blood and from  $0.83 \pm 0.08$   $\mu\text{g/g}$  to  $2.86 \pm 0.28$   $\mu\text{g/g}$  in muscles of Sai CYPRINUS CARPIO table-1.

In Gangetic CYPRINUS CARPIO the Chromium ranged table-2 BDL to  $0.30 \pm 0.10$  ppm in blood and from  $4.32 \pm 0.34$   $\mu\text{g/g}$  to  $16.84 \pm 0.68$   $\mu\text{g/g}$  in muscles. Chromium concentration was highest in muscle of carnivorous CYPRINUS CARPIO C. striatus ( $16.84 \pm 0.68$   $\mu\text{g/g}$ ).

**Manganese (Mn):** In river CYPRINUS CARPIO manganese ranged from  $0.19 \pm 0.06$  ppm to  $0.88 \pm 0.14$  ppm in blood and from  $0.43 \pm 0.17$   $\mu\text{g/g}$  to  $2.67 \pm 0.37$   $\mu\text{g/g}$  in muscles. Highest concentration of Manganese was observed in muscles of omnivorous fish C. batrachus ( $2.67 \pm 0.37$   $\mu\text{g/g}$ ).

In Gangetic CYPRINUS CARPIO the concentration manganese table-2 was from  $0.34 \pm 0.08$  ppm to  $1.88 \pm 0.26$  ppm in blood and from  $7.65 \pm 0.44$   $\mu\text{g/g}$  to  $19.56 \pm 0.49$   $\mu\text{g/g}$  in muscles. Highest concentration of manganese was in muscles of herbivorous fish L. rohita ( $19.56 \pm 0.49$   $\mu\text{g/g}$ ).

**Nickel (Ni):** In river CYPRINUS CARPIO the range of Nickel was recorded from  $0.27 \pm 0.07$  ppm to  $0.44 \pm 0.12$  ppm in blood and from  $0.94 \pm 0.18$   $\mu\text{g/g}$  to  $2.24 \pm 0.36$   $\mu\text{g/g}$  in muscles. Highest concentration of Nickel was observed in muscles of carnivorous fish C. striatus ( $2.24 \pm 0.36$   $\mu\text{g/g}$ ).

In Gangetic CYPRINUS CARPIO Ni ranged table-2 from  $0.21 \pm 0.07$  ppm to  $0.58 \pm 0.09$  ppm in blood and from  $4.83 \pm 0.67$   $\mu\text{g/g}$  to  $12.44 \pm 0.31$   $\mu\text{g/g}$  in muscles. Highest concentration of Nickel was observed in fish C. striatus ( $12.44 \pm 0.31$   $\mu\text{g/g}$ ) in muscles.

**Lead (Pb):** In river CYPRINUS CARPIO the lead ranged table-1 in between  $0.03 \pm 0.01$  ppm to  $0.21 \pm 0.09$  ppm in blood and from  $0.17 \pm 0.05$   $\mu\text{g/g}$  to  $1.04 \pm 0.11$   $\mu\text{g/g}$  in muscle. Highest concentration of lead was observed in fish C. striatus ( $1.04 \pm 0.11$   $\mu\text{g/g}$ ) in muscles.

In Gangetic CYPRINUS CARPIO the lead ranged table-2 in between  $0.05 \pm 0.01$  ppm to  $13 \pm 0.04$  ppm in blood and from  $0.17 \pm 0.03$   $\mu\text{g/g}$  to  $0.49 \pm 0.06$   $\mu\text{g/g}$  in muscles. Carnivorous fish C. striatus show highest concentration of Pb in its muscles ( $0.49 \pm 0.06$   $\mu\text{g/g}$ ).

**Zinc (Zn):** The concentration of Zn in river Sai CYPRINUS CARPIO was table-1 in range from  $4.38 \pm 0.22$  ppm to  $12.21 \pm 0.34$  ppm in blood and from  $12.21 \pm 0.34$   $\mu\text{g/g}$  to  $14.66 \pm 0.55$   $\mu\text{g/g}$  in muscles. Highest concentration of Zinc was observed in muscles of omnivorous fish C. batrachus ( $14.66 \pm 0.55$   $\mu\text{g/g}$ ).

In Gangetic CYPRINUS CARPIO Zinc ranged from  $4.22 \pm 0.19$  ppm to  $14.92 \pm 0.86$  ppm in blood and from  $19.42 \pm 1.49$   $\mu\text{g/g}$  to  $41.06 \pm 4.26$   $\mu\text{g/g}$  in muscles table-2. Highest concentration of zinc was observed in muscles of carnivorous fish *C. striatus* ( $41.06 \pm 4.26 \mu\text{g/g}$ ).

On the basis of above finding it is clear that Zinc concentration was highest in blood and muscle tissue than rest all trace metals in all the three CYPRINUS CARPIO in both the rivers. Carnivorous fish *C. striatus* show higher concentration of much of above mentioned toxic elements than herbivorous fish *L.rohita* and omnivorous fish *C. batrachus* in both the rivers. Concentrations of different trace metals in muscles tissue are higher than concentration observed in blood except few findings. In CYPRINUS CARPIO of both the rivers the concentration of different trace metals varied at different sampling stations. In Sai CYPRINUS CARPIO the concentration of Cr, Mn, Ni, Zn is always higher than those of Cu, Cd and Pb in blood and muscles both in all sampling stations. The pattern of distribution of different trace metals in blood and muscle CYPRINUS CARPIO was

$\text{Zn} > \text{Mn} > \text{Cr} > \text{Ni} > \text{Cd} > \text{Pb} > \text{Cu}$  and  $\text{Zn} > \text{Ni} > \text{Mn} > \text{Cr} > \text{Cu} > \text{Pb} > \text{Cd}$  respectively. The trace metal concentration pattern in Gangetic CYPRINUS CARPIO was  $\text{Zn} > \text{Mn} > \text{Ni} > \text{Cd} > \text{Cr} > \text{Pb} > \text{Cu}$  in blood and  $\text{Zn} > \text{Cu} > \text{Mn} > \text{Cr} > \text{Ni} > \text{Cd} > \text{Pb}$  in muscles. Except Pb rest all above mentioned trace metals are higher in muscles of Gangetic CYPRINUS CARPIO .

### Results and Discussion

The maximum permissible concentration of Cd, Cu, Cr, Mn, Ni, Pb and Zn in food according to various nations such as mentioned by Gopalan et.al<sup>17</sup>, Reilly.<sup>18</sup>, Robert.<sup>19</sup>, W.H.O.<sup>20</sup> have been presented. Our finding for the concentration of Cd in fish muscles (edible part) were always lower than those maxima permitted by Australia and Tasmania. Cu concentration in our results somethings exceeded the maximum permissible limit in food as permitted by Australia, New Zealand, South Africa and U.K. but always lower than those of Canada table-3. When these observed value of Cu were compared with data of “Nutritive value of Indian food”. The Cu concentration was always higher in all observations mentioned by Kousar S., Javed M. and Shuhaimi-Othman M. et. al.<sup>21,22</sup> and recommended maximum permissible concentration of Cr should be 0.01-0.2 mg/kg in food. Our results for Cr concentration in

were higher than this recommendation. Mn concentration in present work sometimes exceeded the recommendations of Gopalan C et.al.<sup>18</sup> but always higher than those maxima permitted by Australia in food. Nickel values observed in present studies were always higher than those maxima permitted in fish products table-3, Pb values in present work were always lower than maxima permitted by Australia, Canada, NewZealand and South Africa in food<sup>23</sup>. Zn values in present work sometimes exceeded the maxima permitted by Australia, and New-Zealand in food but always lower than those of Canada and South Africa<sup>24</sup>.

Mn and Zn contents of muscles of Sai CYPRINUS CARPIO are higher than reported values of river Cauvery CYPRINUS CARPIO except Zn rest all above mentioned metals are lower in muscles than reported by Rupareliaet.al.<sup>5</sup>, in lakeKankaria CYPRINUS CARPIO . Concentration of Zn and Cr is lower than those reported byPandey et.al.<sup>8</sup> in fish muscle of sewage fed pond.

The values of metal concentration in muscles are always higher than values found in blood with some exceptions. This is due to that blood act as distributing medium to whole body and toxic metal search to muscles and other organs via this medium and accumulate there. When these observed values for the concentration of different trace metals in blood in present work were compared with those of terrestrial animals table-4 then it was found that Pb concentration was higher while Cd and Mn concentration were lower than those of reported values in blood of birds of Lucknow city by HussainandKaphalia<sup>10</sup>. Cd, Cu, Cr, Mn, Pb concentration in blood of CYPRINUS CARPIO in the present study were lower while Zn concentration was higher than those reported values in blood of Lizards by Srivastava<sup>9</sup>. Cd value in blood in present work was lower while Cu and Pb values were higher than reported values in blood of Beef steers by Comerford<sup>11</sup>. Pb concentration was higher in blood of Raccoons than observed Pb values in blood of CYPRINUS CARPIO in present work table-2.

**Table-4**

**Average levels of some trace metals in blood of Lizards, Aves, Mammals and CYPRINUS CARPIO**

Animals	Cd	Cu	Cr	Mn	Ni	Pb	Zn	References
Lizards	0.62	0.95	9.14	1.24	-	1.4	1.93	9
Aves	0.07	-	-	0.60	-	1.42	-	10
Mammals	0.02	0.08	-	-	-	0.20	-	11
Beef steers Raccons	-	-	-	-	-	0.44	-	12

CYPRINUS								
CARPIO average								
Values in present work	0.20	0.07	0.25	0.69	0.35	0.10	8.0	

\*All values are in\*- = Data not  
ppm available

### Conclusion

Most of the above toxic metals show higher concentration in carnivorous CYPRINUS CARPIO (C.striatus) than those of herbivorous fish (L. rohita) and omnivorous fish (C. batrachus) in both rivers. This may be due to that carnivorous fish represent highest trophic level hence have high biomagnifications factor for various toxic metals.

Present studies revealed that except Pb rest all above (Cd, Cu, Cr, Mn, Ni, Zn) trace metals are higher in muscles of Gangetic CYPRINUS CARPIO than Sai CYPRINUS CARPIO . This result indicates that fish of river Sai are more suitable for human consumption than Gangetic CYPRINUS CARPIO , River Ganga have a very high degree of contamination yet after GANGA ACTION PLANE.

### References

1. Nagaraju B., Sudhakar P., Anitha A., Haribabu G., and Rathnamma V.V., Toxicity Evaluation and Behavioral Studies of Fresh Water Fish Labeorohita Exposed to Rimon., Int. Jr. Res. Pharma. Biomed. Sci., **2(2)**, 722-727 (2011)
2. Clarkson T.W., Friberg L., Nordberg G.F. and Sager P.R.(ed)., Biological monitoring of toxic metals: New York: Plenum Press, 547-565 (1988)
3. Kutsuna M., Minamata Disease : Study group, Kumamoto Univ. Japan, (1968)
4. Murata I., Hirano T., Saeki Y. and Nakagawa S., Bull. Soc. Lnt. Chrir., **2**, 34 (1970)
5. Ruparelia S.G., VremaY., Pandaya C.B., Sathawara N.G., Shah G.M., Parikh D.J. and Chatterjee B.B., Env. and Eco., **2**, 294-296 (1987)
6. Srivastava P., Faunistic Survey of River Ganga Between Kalakankar and Phaphamau with Special Reference to CYPRINUS CARPIO , Ph.D. thesis : University of Kanpur, (1993)

7. Ayyadurai K, Swaminathan C.S. and Krishaswamy V I.J.E. Hlth., **36(2)**, 99–103 (1994)
8. Pandey B.K., Sarkar U.K., Bhowmik M.L. and Tripathi S.D., J. Env. Biol., **16(2)**, 97-103 (1995)
9. Srivastava K.N., A study of the heart and blood of two species of reptiles. Ph.D. thesis, University of Lucknow,(1983)
10. Husain M.M. and Kaphalia B.S., J. Env. Biol., **11(2)**, 193-201 (1990)
11. Comerford J.W., Bull.Env. Contam. Toxicol., **49**, 18-22 (1992)
12. Hamir A.N, AGalligan D.T., Fbel J.G., Manzell K.L. and Rupprecht C.F., J. Wild. L. Dis., **30(1)**, 115- 118 (1994)
13. Vajpae P. and Sinha A.K., Proc. Symp. Env. Haz. In Patna and Amethi, Patna. F.G.College, 29-34 (1986)
14. Singh R.P., Tayal S, Sinha A.K. and Srivastava R.C., Patna and Amethi., A profile, Symposium on environmental hazards in Amethi and Patna., F.G. College, proceedings., 11-17 (1986)
15. Parkin Elemer Manual., Analytical method for atomic absorption Specetrophotometer, USA : Norkwalk, Connectiuj, (1976)
16. Bais U.E. and Lokhande M.V., Effect of cadmium chloride on histopathological changes in the freshwater fish *Ophiocephalusstriatus* (Channa), International Journal of Zoological Research, **8(1)**, 23-32 (2012)
17. Gopalan C, Ramashashtree B.V. and Balasubramanyan S.C.(ed.), Nutritive Value of Indian foods. Hyderabad : NIN Offset Press ICMR, (1987)
18. Reilly C. (ed.), Metal contamination of food : 56-60, London: Applied Science Publishers Ltd (1980)
19. Robert H.R.(ed.), Food Safety, New York : John Willey and Sons (1981)
20. W.H.O., Health and Safety guide, Geneva: WHO, **62**, 43-44 (1991)
21. Kousar S. and Javed M., Evaluation of acute toxicity of copper to four fresh water fish species, Int. J. Agric. Biol., **14**, 801–804 (2012)
22. Shuhaimi-Othman M, Nadzifah Y. and Ahmad A.K., Toxicity of Copper and Cadmium to Freshwater CYPRINUS CARPIO . World Academy of Science, Engineering and Technology, 65 (2010)

- 23.** Askari Hesni M, Dadolahi-Sohrab A., Savari A. and Mortazav M.S., Study the Acute Toxicity of Lead Nitrate Metal Salt on Behavioral Changes of the Milkfish (Chanoschanos), *World J. Fish and Marine Sci.*, **3(6)**, 496-501 (2011)
- 24.** Ebrahimipoure M., Alipour H. and Rakhshah S., Influence of water hardness on acute toxicity of copper and zinc on fish, *Toxicol. Indus. Health.*, **26**, 361–365 (2010)
- 25.** Sinha A.K., Srivastava K. and Srivastava K.N., *I.J.E.P.*, 888-890 (1994).