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Effect of heavy metal, copper sulphate and potassium chromate on behaviour of "Tailless water flea" *Simocephalus vetulus* (Crustacea - Cladocera)

Anand Mishra[1]*, Sanjive Shukla[2] and A. K. Chopra[1]

[1]Department of Zoology and Environmental Science, Gurukula Kangri University Haridwar-249404 (Uttarakhand), INDIA
[2]P.G. Department of Zoology, B.S.N.V.P.G. College, Lucknow-226001 (U.P.), INDIA
*Corresponding email: anand.mishra.am@gmail.com

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Abstract: Abstract: Water fleas constitute major zooplankton population of fresh water aquatic ecosystem. Their population density is an indicative of well-being of aquatic bodies. *Simocephalus vetulus* (Crustacea - Cladocera) is a tailless water flea and is well suited lab model for environmental monitoring. Copper a Gray listed heavy metals despite being an essential micronutrient, becomes highly toxic when present in excess quantity in aquatic ecosystem thereby causing deleterious effects on aquatic flora as well as fauna. The water-flea exposed to acute 0.37 mg/l (96hr LC_{50}), sub-acute 0.0925 mg/l (25% of 96hr LC_{50}) value and chronic 0.037 mg/l (10% of 96hr LC_{50}) value of copper sulphate and acute 0.16 mg/l (96 hr LC_{50}), sub-acute 0.04 mg/l (25% of 96 hr LC_{50}) value and chronic 0.016 mg/l (10% of 96 hr LC_{50}) value of potassium chromate showed behavioural alterations like initial hyperactivity, fast appendage movements and in phototaxis, geotaxis and avoidance indices. At later stage erratic swimming and spinning, reduced activity, loss of balance, reduced feeding and darkening of cuticular coloration, reduced phototactic, geotaxis and avoidance indices were the major effects on its behaviour. The behavioural alterations of *S. vetulus* showed the most susceptible and foremost indication of potential toxic effects. Various behavioural parameters, used in present study may serve as better biomarkers about metal toxicity and monitoring of drinking water quality.

Keywords: Behavioural alterations, Chromium, Copper, *Simocephalus vetulus*, Tailless water flea

INTRODUCTION

Recent advancing industrialization and urbanization have increased salt concentrations in formerly fresh water habitats. The freshwater animals are being affected, especially those like crustaceans that are unable to emigrate to escape the problem. Salinity is an abiotic factor delineating the environmental optimum for freshwater crustaceans (Ghazy *et al*., 2009; Mitra *et al.,* 2010). The freshwater cladocerans are widely used by environmental scientists to monitor drinking water quality because of their quick response to toxins in the water (Ghazy *et al.*, 2009). Cladocerans are group of crustaceans which constitute major zooplankton community of the freshwater aquatic bodies. Among Cladocerans, family Daphniidae is more common consisting mainly of *Daphnia*, *Ceriodaphnia* and *Simocephalus*. These members can be helpful in assessment of environmental conditions and can act as better bioindicators of aquatic pollution. (Rao, 2001; Raghunathan and Kumar, 2003; Lee *et al.*, 2004; Young, *et al.,* 2012 and Mishra, *et al*., 2016).

The behavioural change is a manifestation of the motivational, biochemical, physiological and environmentally influenced state of the organism. Verplanck, (1957) defined ''behaviour'' as all the observable, recordable and measurable activities of living organism. It includes all those process by which an animal sense the external word and the internal states of the body and responds to the change which it perceives (Svoboda, 2001; Witeska, 2005 Lodhi *et al*., 2006; Sharma and Shukla, 2006 and Levitis *et al*., 2009).

Cladocerans (*Ceriodaphnia*, *Daphnia*, *Simocephalus* and *Moina*) are important aquatic organisms because they transfer energy from primary producers to consumers of higher trophic level, such as fish. At the same time, they are commonly used in measuring pollution level, drinking water quality and in testing of human body fluids, due to their macroscopic size, easy culture methods, short life span and parthenogenetic mode of reproduction (Hanazato, 2001; Smirnov, 2014; Arora *et al*., 2014 and Mishra *et al*., 2016).

Simocephalus vetulus, "tailless freshwater flea" are small crustaceans that are less expensive in maintenance, very sensitive to heavy metal toxicity, easy to care and are translucent in colour. The latter property allows to easily observing the behavioural alterations. Considering above facts present work has been turn into account to

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evaluate behavioural alterations of *S. vetulus* after copper and chromium exposure.

MATERIALS AND METHODS

The fresh water "tailless water flea", *S. vetulus* were collected from the freshwater pond located near Ikkar railway station at Haridwar (Uttarakhand) and their culture was maintained by the standard method (Davis and Ford, 1992).

Preparation of toxicant and analysis of physico-chemical parameters: Stock solutions of Copper sulphate ($CuSO_4.5H_2O$) and Potassium chromate (K_2CrO_4) both of AR: Merck Specialities Private Limited, Mumbai, India) were prepared by dissolving weighed amount (mg/l) of salt in double distilled water. Two to three drops of glacial acetic acid was added to stock solution of copper sulphate so as to prevent the precipitation. The experimental medium was analyzed for its physico-chemical parameters (Temperature, pH, Dissolved oxygen and Total hardness) as per standard methods (APHA *et al.*, 2012).

Experimental set up: All behavioural tests were carried out on neonates (age < 24 hr) obtained by isolating the adults from the stock culture. All the behavioural alteration was recorded for the concentration of acute 0.37 mg/l (96hr LC_{50}), sub-acute 0.0925 mg/l (25% of 96hr LC_{50}) value and chronic 0.037 mg/l (10% of 96hr LC_{50}) value of copper sulphate and acute 0.16 mg/l (96 hr LC_{50}), sub-acute 0.04 mg/l (25% of 96 hr LC_{50}) value and chronic 0.016 mg/l (10% of 96 hr LC_{50}) value of potassium chromate.

Behavioural alterations, like phototaxis, geotaxis and avoidance, hyperactivity, fast appendage movements, swimming and spinning, reduced activity, reduced feeding and cuticular coloration, were carefully observed in experimental animals and compared with control ones.

For phototaxis, light exposure of 2735 lm was used with Philips 45 watts CFL for fixed duration. The observations were replicated thrice for ten individuals each at all exposures and the data were subjected to statistical analysis using statistical software on PC. Phototactic, geotaxis and avoidance indices were calculated ranging from -1 to +1 following the standard methods described by (Whitman and Miller, 1982; De Meester, 1993; Decaestecker *et al*., 2002).

Statistical analysis: Data of behaviour of *S. vetulus* obtained from different experiments in replicates were compared to control. The trends of data in experimental and control were statistically analyzed by comparison of means using statistical software Minitab and supporting software like MS-excel on PC.

RESULTS

Acute toxicity on general behaviour patterns: Acute toxicity tests carried out on 96 hr LC_{50} value of $CuSO_4.5H_2O$ and K_2CrO_4 to evaluate the subsequent behavioural effects of *S. vetulus* at 24, 48, 72 and 96 hr of exposure showed that after acute exposure (0.37 mg/l) of $CuSO_4.5H_2O$ induced marked alteration on general behavioural patterns of *S. vetulus* were observed. Exposed animals showed hyper activity at initial stage followed by erratic swimming and gradually loss of balance in almost all the animals.

Darkening was observed maximum in carapace and head region in of the animals. In some animals,cloudy droplets appeared under carapace. In initial stage, animal showed aggressiveness and after that loss of aggressive behaviour was observed. In initial stage mucous secretion was not observed in *S. vetulus* after 72 and 96h of exposure, however very less amount of mucous secretion was observed in 10% of animal population. Food detection and consumption of *S. vetulus* was found normal in initial stage and considerably declined after that 48, 72 and 96h exposure of copper sulphate (Table 1; Plate 1).

Potassium chromate induced marked alteration on general behavioural patterns. After introduction *S. vetulus* showed hyper activity at initial stage followed by erratic swimming and gradually lass of balance was observed in almost 75% of the animals. Rough surface of carapace was observed in about 55% of the animals. In initial stage *S. vetulus* showed aggressive behaviour and after that loss of aggressive behaviour was observed. In initial stage mucous secretion was not observed in *S. vetulus* but after 96h of exposure mucous secretion was observed in 20% animals. Food detection and consumption of *S. vetulus* was found normal in initial stage and after that 48, 72 and 96h exposure of potassium chromate it declined considerably (Table 2; Plate 2).

Sub-acute and chronic toxicity effect on behaviour: The behavioural observations in terms of phototaxis, geotaxis and avoidance at 7, 14 and 21days of sub-acute and chronic exposures are mentioned in Figs. 1 to 6.

Effect on phototaxis: Copper sulphate induced marked alteration on phototactic behaviour of *S. vetulus*. Phototactic index was found decreased in sub-acute copper sulphate exposure after 7, 14 and 21 days exposure (0.72, 0.7 and 0.62) in comparison to control (0.86, 0.8 and 0.8). While similar decreased was noticed in chronic copper sulphate exposure after 7, 14 and 21 days exposure (0.78, 0.76 and 0.7) in comparison to controls (0.86, 0.8 and 0.8). The phototactic index of control and experimental animal after exposure of copper sulphate is given in (Fig. 1).

Phototactic index was found decreased in sub-acute potassium chromate after 7, 14, and 21 days exposure (0.7 to 0.64) in comparison to control (0.80 to 0.76). While similar decrease was noticed in chronic potassium chromate after 7, 14 and 21 days exposure (0.78, 0.7 and 0.66) in comparison control (0.82 to

Table 1. Effect of copper sulphate on behavioural parameters of fresh water 'tailless flea', *S. vetulus* after acute exposure.

S.N.	Exposure Duration (hrs)	Group	Behavioural Responses						
			1	2	3	4	5	6	7
		Control	N	N	N	N	N	N	N
1.	24	Experimental	H+	E+	B+	N	A+	NS	N
		Control	N	N	N	N	N	N	N
2.	48	Experimental	H+	E+	B+	D	A+	NS	+
		Control	N	N	N	N	N	N	N
3.	72	Experimental	H-	E+	B-	D	++	+	+
		Control	N	N	N	N	N	N	N
4.	96	Experimental	H-	E+	B-	+D	++	+	+

1- Activity; 2- Swimming style; 3- Balance; 4- Coloration; 5- Aggression; 6-Mucous secretion; 7- Food detection and consumption. H+= Hyper activity, H-= Hypo activity, E+= Erratic swimming, B+= Stable, B-= Unstable, D=Dark; +D=Very dark, A+= aggressive, A-= Non-aggressive, N=Normal; - = Nil, + = Less, ++ = More, NS = No secretion.

Table 2. Effect of potassium chromate on behavioural parameters of fresh water 'tailless flea', *S. vetulus* after acute exposure.

S.N.	Exposure Duration (hrs)	Group	Behavioural Responses						
			1	2	3	4	5	6	7
		Control	N	N	N	N	N	N	N
1.	24	Experimental	H+	N	B+	+	+	+	++
		Control	N	N	N	N	N	N	N
2.	48	Experimental	H+	E+	B+	+	+	+	+
		Control	N	N	N	N	N	N	N
3.	72	Experimental	H-	E+	B-	+	++	NS	+
		Control	N	N	N	N	N	N	N
4.	96	Experimental	H-	E+	B-	+	++	+	-

1- Activity; 2- Swimming style; 3- Balance; 4- Coloration; 5- Aggression; 6-Mucous secretion; 7- Food detection and consumption. H+= Hyper activity, H-= Hypo activity, E+= Erratic swimming, B+= Stable, B-= Unstable, D=Dark; +D=Very dark, N=Normal; - = Nil, + = Less, ++ = More, NS = No secretion.

0.67). The phototactic index of control and experimental animal after exposure of potassium chromate is given in (Fig. 4).

Effect on geotaxis: *S. vetulus* showed decrease in geotaxis index after sub-acute and chronic exposure of copper sulphate after 7, 14 and 21days. After 7 day the geotaxis index was calculated as 0.76 for sub-acute and 0.56 for chronic which was significantly lower than the control value (0.96).

After 14 and 21days the geotaxis index was estimated as 0.82 and 0.88 for high concentration and 0.66 for low concentration of copper sulphate which was less than the control value (1 after 14days 0.96 after 21 days). The geotaxis was also affected by the concentration of the toxicant as it was found directionally proportional to the concentration for all exposure duration (Fig. 2).

Potassium chromate induced marked alteration on geotaxis behaviour of *S. vetulus*. Geotaxis index was found increased in sub-acute from 7, 14, and 21 days of exposure (0.76, 0.8 and 0.84 respectively) but the estimated values was less than the control ones (0.92, 0.94 and 0.96).

A slight increased in geotaxis response was observed in chronic potassium chromate after 7, 14, and 21 days exposure (0.58, 0.64 and 0.7) in comparison to control. The geotaxis index of control and experimental animal after exposure of potassium chromate is given in (Fig. 5).

Effect on avoidance: Copper sulphate induced marked alterations on avoidance behaviour of *S. vetulus*. Avoidance index was found decrease in sub-acute exposure after 7, 14 and 21 days exposure (0.96, 0.94 and 0.90) in comparison to control (1) and similar decreased was noticed in chronic exposure after 7, 14 and 21 days exposure (0.88, 0.86 and 0.80) in comparison to control (1).

The avoidance was also affected by the concentration of the toxicant as it was found directionally proportional to the concentration for all exposure duration. The avoidance index of control and experimental animals after exposure of copper sulphate is given in (Fig. 3).

The avoidance index was found decrease in sub-acute potassium chromate after 7, 14 and 21 days exposure (0.94, 0.92 and 0.88) in comparison to control (1). A slight decreased in chronic potassium chromate after 7, 14 and 21 days exposure (0.9, 0.86 and 0.82) in comparison to control (1). The avoidance index of control and experimental animal after exposure of potassium chromate is given in (Fig. 6).

DISCUSSION

Behavioural toxicology is a tool for hazard assessment of water pollution (Beitinger and freeman, 1983). Behavioural changes in animals are indicative of internal

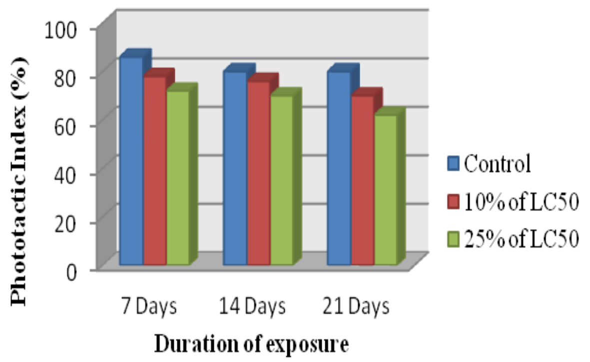

Fig. 1. *Effects of copper sulphate on phototaxis behaviour of fresh water 'tailless flea', S. vetulus after sub-acute and chronic exposure.*

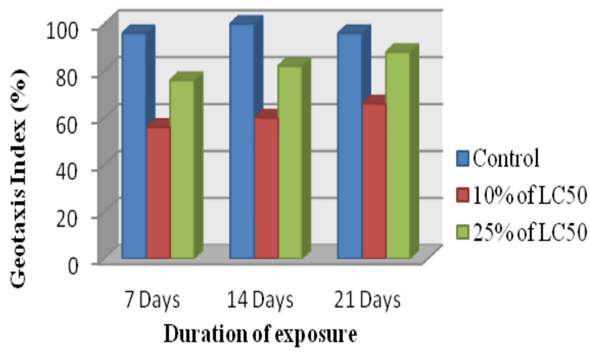

Fig. 2. *Effects of copper sulphate on geotaxis behaviour of fresh water 'tailless flea', S. vetulus after sub-acute and chronic exposure.*

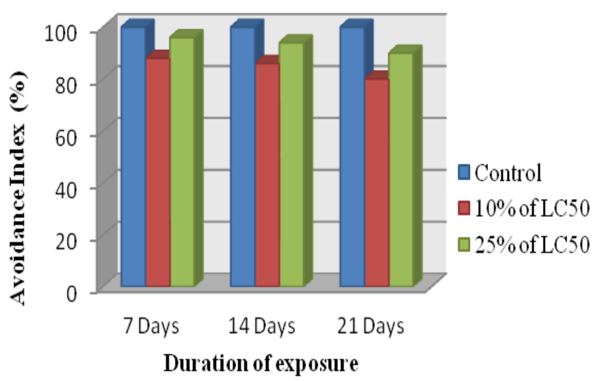

Fig. 3. *Effects of copper sulphate on avoidance behaviour of fresh water 'tailless flea', S. vetulus after sub-acute and chronic exposure.*

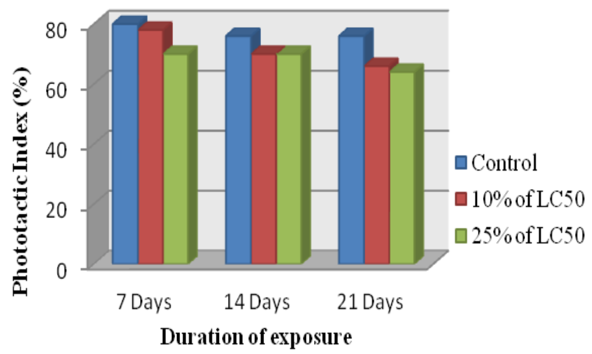

Fig. 4. *Effects of potassium chromate on phototaxis behaviour of fresh water 'tailless flea', S. vetulus after sub-acute and chronic exposure.*

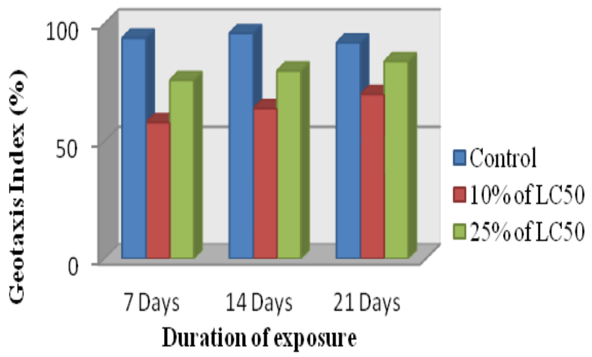

Fig. 5. *Effects of potassium chromate on geotaxis behaviour of fresh water 'tailless flea', S. vetulus after Sub-acute and chronic exposure.*

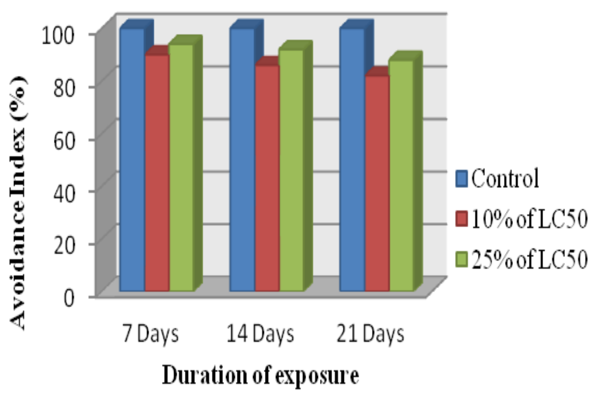

Fig. 6. *Effects of potassium chromate on avoidance behaviour of fresh water 'tailless flea', S. vetulus after sub-acute and chronic exposure.*

disturbance of body functions such as inhabitation of enzyme function, impairment in neural transmission and disturbance in metabolic pathway (Shah, 2002). Disruption of physiological process and neurological effects being late are the manifestations of pollution. Need has continuously been felt to identify non-invasing types of sensitive bio-indicators for early detection, warning and monitoring of the presence of pollutions in the environment (Masud *et al*., 2005). The behavioural responses to pollutants are considered to have the potential for serving the purpose of biomarkers (Masud *et al*., 2005). In presnt study, various behavioural parameters of *S. vetulus* such as activity, swimming style, balance, coloration, aggression, mucous secretion,

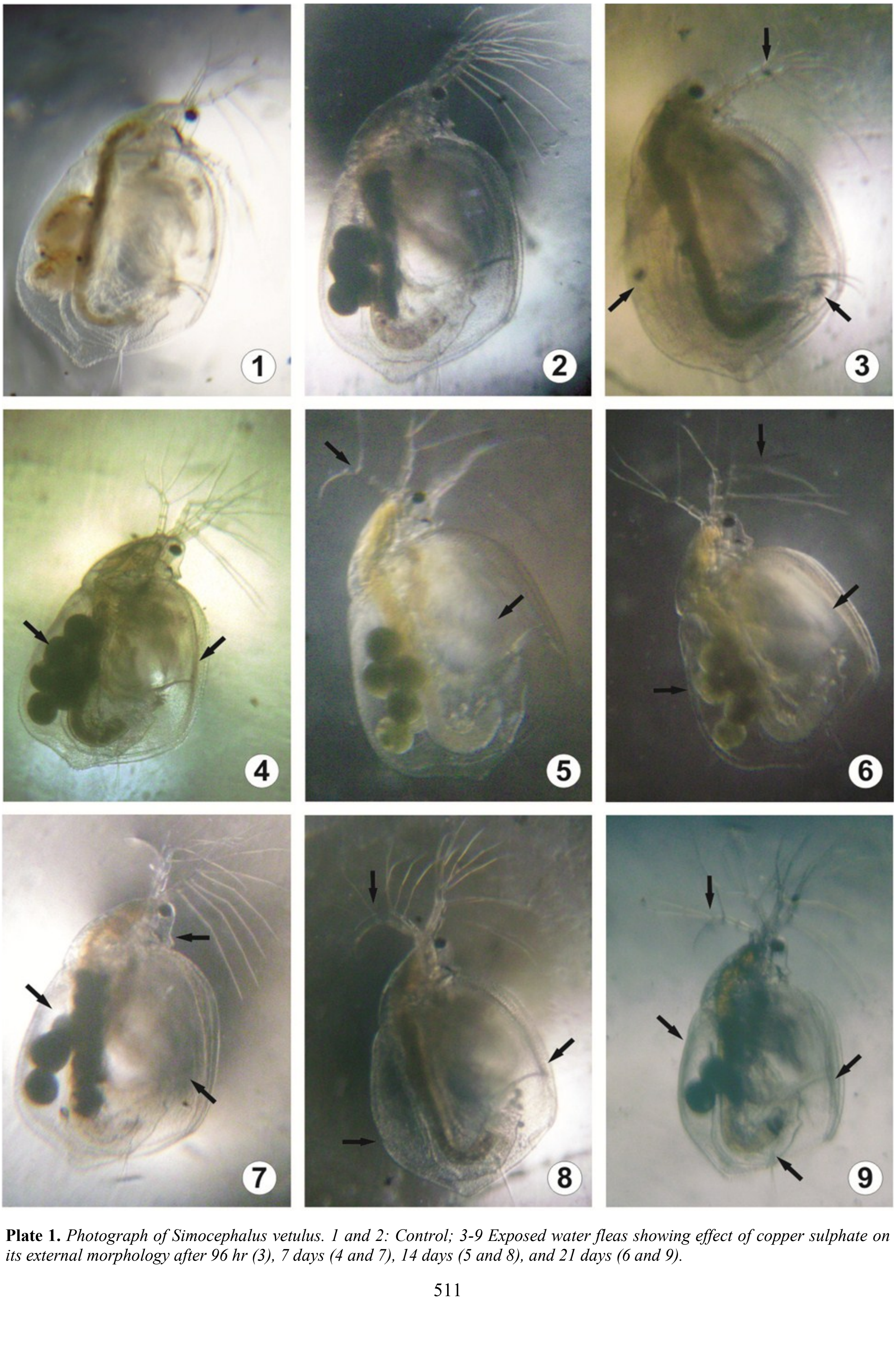

Plate 1. *Photograph of Simocephalus vetulus. 1 and 2: Control; 3-9 Exposed water fleas showing effect of copper sulphate on its external morphology after 96 hr (3), 7 days (4 and 7), 14 days (5 and 8), and 21 days (6 and 9).*

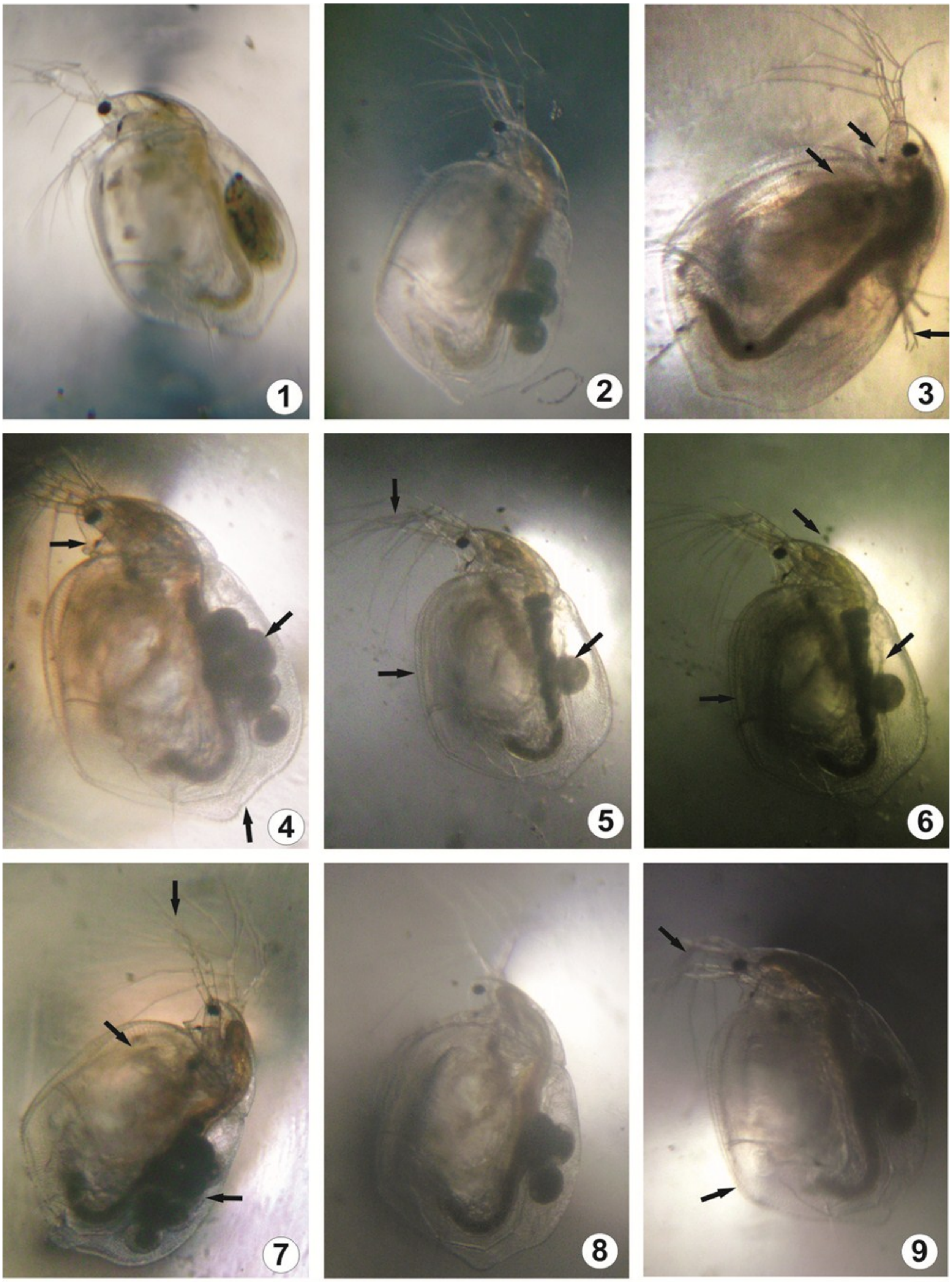

Plate 2. *Photograph of Simocephalus vetulus. 1- 2: Control; 3-9: Exposed water fleas showing effects of potassium chromate on its external morphology after 96 hr (3), 7 days (4 and 7), 14 days (5 and 8) and 21 days (6 and 9).*

food detection and consumption, phototaxis, geotaxis and avoidance showed changes/fluctuations after exposure of copper sulphate (Table 1 and Figs. 1, 2, and 3) and potassium chromate (Table 2 and Figs. 4, 5 and 6). The copper sulphate imposed significant effect on behaviour during various toxic exposures like acute exposure of copper sulphate, marked alteration on general behavioural patterns were observed. After introduction of *S. vetulus*, it showed hyper activity at initial stage followed by erratic swimming, lass of balance, darkening of carapace, loss of aggressive behaviour, mucous secretion, food detection and consumption of *S. vetulus* was found normal in initial stage and considerably declined after that 48, 72 and 96h exposure of copper sulphate.

In initial stage, acute exposure of potassium chromate induced marked alteration on general behavioural of *S. vetulus* showing hyperactivity, erratic swimming and gradually loss of balance was observed in almost 75% of the animals. Rough surface of carapace, loss of aggressive behaviour, mucous secretion, reduction in food detection and consumption were observed. Almost similar observations have been reported on *D. magna* and *D. magna straus* after exposure of industrial effluents by various workers (Ren *et al*., 2009; Kovacs *et al*., 2012; Terra and Gonçalves, 2013; and Jeong *et al*., 2014) as well as on the other crustaceans (Macedo-Sousa, 2007 and Wiklund *et al*., 2011). Irratic swimming, loss of balance during swimming might be due to some neurological impairment in central nervous system as evident by inhibition of AChE by cadmium and other metals (Patro, 2006).

In aquatic organisms, behaviour is considered a sensitive indicator of chemically induced stress (Sharma and Shukla, 1990; Roast *et al*., 2000). Stress induced by changes in the environmental parameters during handling and transportation require homeostatic regulation which brings about behavioural and physiology alteration in aquatic animals (Lorenzon, 2005).

The behavioural symptoms like initial hyperactivity, increased appendage movement, erratic swimming, darkening of carapace and all over the body and blackening of carapace margin and dorsal carapace region, loss of balance, letharginess and bottom setting was noticed up to 96 hour of exposure to acute concentration of metals. Almost similar observations were recorded by Vijayaraman *et al*., (1999) on *M. malcolmsonii*; Verslycke *et al*., (2003) on Mysid, *Neomysis integer* and Sharma and Shukla (2006) on *M. lamarrei*. Toxic effects appear beyond an organism's resilience range like hyperactivity leading to exhaustion, decreased activity and finally lethality.

Excitement, hyperactivity and abnormal jerky swimming observed in *Heteropneustes fossilis* and *Arius nenga* may be caused by accumulation of neurotransmitter in neuromuscular junctions (Pandey *et al*., 2009; Singh *et al*., 2009 and Sreelekshmy *et al*., 2016).

Locomotion may be considered as an integration of physiological, sensory, nervous and muscular systems in *Brachionus* (Charoy *et al*., 1995). The initial hyperactivity may be an avoiding reaction to the heavy metals as it is evident that animals tend to avoid and counter react to various irritants and toxicants. Similar finding have also been observed in fishes after the exposure of other heavy metals like Pb, Cu, Ni, Zn and Hg (Black and Binge, 1989). Sornraj *et al*., (1995) studied similar observation in *C. punctatus* after and exposure of Cr, Ni, and Zn. Behavioural alterations have been also studied in crustaceans after exposure of various toxicants (Sharma and Shukla, 1990). Toxicity induced decreased activity and surfacing may be as a result of altered carbohydrate metabolism (Radhakrishnaiah and Bussapa, 1986) or even may be due to alterations in muscle structure and coordination (Schultz and Kennedy, 1977).

In present study, mucus secretion observed after copper sulphate and potassium chromate exposure in *S. vetulus* may be due to irritation caused by metallic ions as observed by (Sharma and Shukla, 1990 and Verma *et al*., 2010). Mucous secretion on carapace and almost whole body surface observed in the present study is a normal response to toxic chemicals and irritants. It may be a good defensive mechanism to inhibit the uptake of the chemical through the gills and combat its toxic effect. Similar pattern of mucus secretions was also observed by Nagabhushnam and Sharojini, (1989) in *M. lamarrei, M. kistenensis* and *Scylla Serrata*; Sharma and Shukla, (1990) in *M. lamarrei*. However, excessive mucous secretion can cause mucous coagulation and precipitation with metals on the body surface, ultimately resulting in death of organism due to suffocation (Plonka and Neff, 1969). Increased mucous secretion probably helps in countering irritating effect of toxicant in skin and mucous membrane. The blackening of carapace and post abdominal claw due to exposure of toxicant were very pronounced in the present investigation (Plates 1 and 2). The gill blackening has been reported in *P. duroronum*, *Palaemonetes pugio*, *P. vulgaris* after cadmium chloride exposure (Nimmo *et al*., 1977 and Verma *et al*., 2010); in *Caridina rajdhari* after $CuSO_4.H_2O$ exposure (Ghate and Mulherker, 1979), in *P. pugio* after hexavalent chromium exposure(Doughtie *et al*., 1983). in *C. rajdhari* and *M. kistenensis* after mercury and cadmium exposure (Ghate, 1984). The probable reason for blackening may be due to cell necrosis in the gill tissue (Nimmo *et al.,*1977), while Ghate, (1984) opined that deposition of melanin caused blackening of the gills.

Heavy metals influence a broad spectrum of physiological processes in organisms particularly the nervous system. The blocking effect of copper with receptor membrane or enzyme components has been postulated by electroencephalographic recording in

Salmon fish (Hara, 1972). Elevated level of copper caused a decrease of the encephalographic response from the olfactory bulb of salmon thus impairing its sense of odour. It is evident that swimming, coordination of movements, Orientation aggregation are affected by metal pollution in crustaceans (Roast *et al*., 2000, 2002) as well as in fishes (Jezierska *et al*. 2002 and Drastichova *et al.,* 2004). Inhibition of acetycholineosterase, an essential enzyme for synaptic transmission, has been reported in crayfish, *Procambarus clarkii* after Hg, Cd and Pb exposure (Devi and Fingerman, 1995).

Effect on phototaxis: *S. vetulus* is positively phototactic in normal conditions. The phototactic index was found continuously decreased after increasing concentration of potassium chromate in sub-acute and chronic exposure duration in after 7, 14, and 21 days exposure (Fig. 4).

Almost similar observations were recorded by Guerra (2001) on *D. magna*; Mahvi *et al*. (2007) on *D. magna*. Kien *et al*. (2001) observed *D. magna* becomes less positively phototactic in presence of 0.02 mg/l cadmium. Similarly $K_2Cr_2O_7$ inhibited phototaxis *D. carinata* (Wu *et al*., 2005). Phototaxis was also found dipressed in *D. magna* by Bacitrain and Lincomycin (Delupis *et al*., 1992) and the findings are in agreement with the findings of present work.

In cladocerans, light intensity triggers vertical migration, changes in photoperiod triggers diapauses. Metals or toxicants affecting the phototaxis may hamper the food searching and consumption thereby enhancing the ecological competition and their survival which in turn reducing the pond productivity. Sprague (1969) suggested that behavioural bioassay may be even more sensitive than survival, growth and reproduction. The phototaxis bioassays proved to be a useful means of demonstrating toxic effects of toxicants to *D. magna* were reported by LeBlanc (1980). *Daphnia*, *Simocephalus* and other cladocerans species are the most sensitive organisms of fresh water aquatic bodies (Mahvi *et al*., 2007; Smirnov, 2014). The higher concentration of toxic chemical and metals that caused behavioural effects of *Daphnia* as well as other cladocerans species (Mahvi *et al*., 2007; Smirnov, 2014 and Luciana *et al*., 2014).

Effect on geotaxis: Taxis is the directional movement, the geotaxis behaviour gets more or less altered when animal exposed to any xenobiotics. *S. vetulus* showed marked alteration on geotaxis behaviour after sub-acute and chronic exposure of copper sulphate, the geotaxis index was found reduced in comparison to control after 7, 14 and 21days. The geotaxis was also affected by the concentration of the toxicant as it was found directionally proportional to the concentration for all exposure duration (Fig 2).

Potassium chromate induced marked alteration on geotaxis behaviour of *S. vetulus*. Geotaxis index was found increased in sub-acute from 7, 14, and 21 days of exposure but the estimated values was less than the control ones. A slight increased in geotaxis response was observed in chronic potassium chromate after 7, 14, and 21 days exposure in comparison to control (Fig. 5).

Almost similar observations on geotaxis behaviour were recorded by Moore *et al*. (2000) on *D. retrocurva*; Rautio *et al*. (2003) on *Daphnia. longispina*; Bauer *et al*. (2005) on *Gammarus pulex* and *G. roeseli*; Sanchez *et al*. (2007) on *Artemia parthenogenetica*.

In aquatic ecosystem, predation risk is believed to be an important factor in determining the vertical position of many species in water column (Hays, 2003). Toxic chemicals present in the water as well as chemicals of predators "Kairomones" affect vertical position and diel migration timing of *Daphnia* and zooplanktons in both fresh water as well as marine system have been well document (Fortier *et al*., 2001 and Irigoien *et al*., 2004). Heavy metals affecting geotactic responses may induce predation risk to these planktons which can affect the productivity of pond.

Effect on avoidance: Present findings after copper sulphate exposure *S. vetulus* showed marked alteration in avoidance behaviour. Avoidance index was found to decrease in sub-acute and chronic copper sulphate exposure after 7, 14 and 21 days exposure in comparison to control. The avoidance was also affected by the concentration of the toxicant as it was found directionally proportional to the concentration for all exposure duration (Fig. 3). The avoidance index was found to decrease in sub-acute potassium chromate after 7, 14 and 21 days exposure in comparison to control. A slight decreased in chronic potassium chromate after 7, 14 and 21 days exposure in comparison to control (Fig. 6).

Almost similar observations on avoidance behaviour were recorded by Lopes *et al*. (2004) on *D. magna*. Behavioural alterations have been established as sensitive indicators of chemically induced stress in aquatic organisms (Sharma and Shukla, 1990 and Agrawal, 1991). Behavioural alterations like avoidance was observed in present study may be an avoiding reaction to the heavy metals as also observed by various workers for crustaceans (Svecevicus, 2001; Sharma and Shukla, 2006; Lodhi *et al*., 2006; Sharma *et al*., 2008; Kasherwani *et al*., 2009 and Seuront, 2010). The avoidance reaction may be related to narcotic effects or to change in sensitivity of chemo receptors of aquatic organisms as reported by Sutlerlin (1974).

Conclusion

The acute exposure of copper sulphate and potassium chromate induced marked alterations on general behavioural patterns of the tailless water flea, *S. vetulus*. The fleas showed hyper activity at initial stage followed by erratic swimming, loss of balance, darkening of carapace, loss of aggressive behaviour, mucous secretion. The food detection and consumption of the flea was found normal in initial stage and considerably declined after that 48, 72 and 96h exposure of copper sulphate and potassium dichromate. The phototactic, geotaxis and avoidance indices were found decreased after exposure of 7, 14 and 21 days exposure of sub-acute and chronic toxicity of copper sulphate and potassium dichromate. The findings of present study may beneficial for future prediction of safe concentration and well being of aquatic bodies. The behavioural parameters of the tailless water flea may serve as better biomarkers in relation to metal toxicity.

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