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Acute Toxicity of Some Agriculture Fertilizers to Rainbow Trout

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Abstract

The acute toxicity of ammonium sulphate, calcium ammonium nitrate and two different composite fertilizers [15:15:15 (NPK-1) and 25:5:10 (NPK-2)] to juvenile rainbow trout (*Oncorhynchus mykiss*; 0.89±0.22 g) was evaluated under semi-static conditions. The concentrations of ammonium sulphate, calcium ammonium nitrate, NPK-1 and NPK-2 that killed 50% of the rainbow trout within 96-h (96-h LC_{50}) were 0.149, 0.259, 0.258 and 0.408 g/L, respectively. The mortality of fish was elevated with increasing concentrations of the fertilizers. The time required to kill 50% (LT_{50}) of the rainbow trout at the highest concentrations tested was 11 h 18 min for ammonium sulphate, 12 h 23 min for calcium ammonium nitrate, 13 h 38 min for NPK-1 and 17 h 47 min for NPK-2. Ammonium sulphate was the most toxic compound to rainbow trout compared to composite fertilizers.

Keywords: fertilizer, NPK, acute toxicity, rainbow trout.

Bazı Tarımsal Gübrelerin Gökkuşağı Alabalıklarına Akut Toksik Etkileri

Özet

Amonyum sülfat, kalsiyum amonyum nitrat ve kompozit gübrelerin [15:15:15 (NPK-1) ve 25:5:10 (NPK-2)] gökkuşağı alabalığı (*Oncorhynchus mykiss*; 0,89±0,22 g) yavruları üzerine olan akut toksik etkileri, 96 saat süreli yarı statik test yöntemi kullanılarak belirlenmiştir. Gökkuşağı alabalıklarının %50'sini 96 saat içinde öldüren amonyum sülfat, kalsiyum amonyum nitrat NPK-1 ve NPK-2 konsantrasyonları sırasıyla 0,242, 0,408, 0,368 and 0,549 g/L'dir. En yüksek toksik konsantrasyona maruz bırakılan gökkuşağı alabalıklarının %50'sinin ölmesi için gereken zaman amonyum sülfat için 11 saat 18 dakika, kalsiyum amonyum nitrat için 12 saat 23 dakika, NPK-1 için 13 saat 38 dakika ve NPK-2 için 17 saat 47 dakika olarak belirlenmiştir. Amonyum sülfatın, kullanılan gübreler içerisinde gökkuşağı alabalıkları üzerine en yüksek toksik etkiye sahip olduğu tespit edilmiştir.

Anahtar Kelimeler: gübre, NPK, akut zehirlilik, gökkuşağı alabalığı.

Introduction

Nitrogen pollution from agricultural sources is now considered to be a major problem in many regions of the word (Haygarth and Jarvis, 2002; Vidal *et al*., 2000). This has originated from human habitation, agriculture and large numbers of farm animals, such as pigs and cows (Randall and Tsui, 2002). Nitrogen (N) presents in fertilizer in two principal forms, organic-N and inorganic-N. Ammonium is the main inorganic form of nitrogen (Clarkson *et al*., 1986). Aquatic systems can have ammonium concentrations high enough to adversely impact fish as a result of wastewater treatment plant discharges, degradation of nitrogen-containing organic matter, fertilizer runoff and industrial sources (Chouhan and Pandey, 1987; Palanivelu *et al*., 2005).

Composite fertilizers (NPK) are not classified as hazardous material according to EEC Directive 67/548/EEC (EPC, 1999). As these fertilizers contain phosphates they may cause adverse environmental impact such as eutrophication in confined surface water. NPK, without urea has low potential for bioaccumulation and low toxicity to aquatic life (EIFAC, 1973; EFMA, 2005).

Concentrations of 0.3 mg inorganic-N/L have been identified as levels above which environmental problems can occur (Gangbazo *et al*., 1995). Accumulation of ammonium in water may lead to decreased growth (Thurston and Russo, 1983; Palanichamy *et al*., 1985), changes fish behavior (Rani *et al*., 1997; Wicks and Randall, 2002) and increased vulnerability to disease (Thurston *et al*., 1984). Furthermore, sublethal ammonium concentrations in water showed inhibitory effects on the enzyme activities of fish (Hisar *et al*., 2004) and caused degeneration on different tissues (Erdogan *et al*., 2005).

The present study aimed to determine the acute toxicity of ammonium sulphate, calcium ammonium nitrate, NPK-1 and NPK-2 to rainbow trout using a static test system. As studies on the lethal times of ammonium sulphate, calcium ammonium nitrate NPK-1 and NPK-2 for rainbow trout in the literature are insufficient.

Materials and Methods

Experimental Animals

Juvenile rainbow trout (*Oncorhynchus mykiss*) (0.89±0.22 g; 4.59±0.33 cm; Mean±SD) were obtained from Karadeniz Technical University, Faculty of Marine Sciences. Fish were held in recirculation systems (200 L) for at least 15 days to acclimate to laboratory conditions prior to experiments. During the acclimation period, about 50% of the water in each recirculation system was replaced daily. Throughout the acclimation period and subsequent periods of fertilizers exposure, fish were held under a photoperiod of 12 hours of light and 12 hours of darkness. Fish were fed commercial trout pellets at 2.5% of their body weight twice a day until 2 days before exposure and not fed during the toxicity tests.

Water Quality

During exposure to fertilizers, temperature, pH, unionized ammonia, nitrite and dissolved oxygen in each treatment were measured daily. Water temperature, pH and dissolved oxygen were determined with a glass electrode (Oxi 330i, WTW, Germany). Total ammonia was measured by an indophenols method and nitrite was measured by an azo method (Boyd and Tucker, 1992).

Experimental Design

The acute toxicity test design was conducted based on guidelines provided by Altinok *et al*. (2006). The fish were determined to be free of external parasites prior to the exposure (AFS-FHS, 2003). After acclimation, fish from one of the acclimation tanks were randomly transferred glass aquaria containing 25 L of static water. Triplicate random groups of 10 fish each were subjected to experimentation for 96 h in semi-static fresh water containing different concentrations of fertilizers.

Triplicate aquaria were designated for each concentration of ammonium sulphate [$(NH4)_2SO_4$; 21% nitrogen as ammonium ions and 24% sulfur as sulfate ions], calcium ammonium nitrate (CAS; $5Ca(NO_3)2NH_4NO_3.10H_2O$; 27% N and 20% of ground limestone), 15:15:15 (NPK-1; 15% potassium, 15% phosphorus and 15% nitrogen) and 25:5:10 (NPK-2 25% nitrogen, 5% phosphorus and 10% potassium). The concentrations tested for ammonium sulphate were 0 (control), 0.025, 0.05, 0.10, 0.20, 0.22, 0.25, 0.27 and 0.30 g/L. The calcium ammonium nitrate concentrations were 0 (control), 0.15, 0.20, 0.25, 0.30, 0.35, 0.40 and 0.50 g/L. NPK-1 concentrations were 0 (control), 0.012, 0.50, 0.10, 0.25, 0.40, 0.55 and 0.70 g/L. NPK-2 concentrations were 0 (control), 0.05, 0.20, 0.35, 0.50, 0.65 and 0.80 g/L. This study was conducted under OECD Guideline No. 203 for static-renewal test conditions (OECD, 1992). Concentrations of fertilizers were re-established to maintain the original levels while fifty percent of the test solution was renewed each day. During the 96 h acute toxicity experiment water in each aquarium was aerated and had the following characteristics: dissolved oxygen 9.04±0.53 mg/L, temperature 16.65±0.44ºC and pH 7.45±0.07. Unionized ammonia and nitrite concentrations at the different concentrations of fertilizers are presented in Table 1.

Statistical Analyses

Statistical analyses were performed after Altinok and Capkin (2007). Briefly, the estimated concentrations of fertilizers that kill 50% of rainbow trout within 96 hours (96-h LC_{50}) and the time required to kill 50% (LT_{50}) were calculated by probit analysis (SPSS 2008, SPSS Inc., Chicago, IL, USA). After exposing the fish to different concentrations of fertilizers, survival of fish were analyzed by Kaplan-Meier survival and failure time analysis tests (KMSFTAT). After analyzing the survival data, significant differences were found ($P<0.05$), (Statistica, Statsoft Inc., Tulsa, Oklahoma, USA).

Results

No fish died during the acclimation period before fertilizers exposure and no control fish died during toxicity tests. Fish exposed to 200 mg/L and above that concentration had a rapid respiration rate, loss of equilibrium, erratic swimming, flashing and crowding at the water surface. After long periods of motionlessness, it was seen that the fish lied down on the aquarium bottom and suddenly started to move. At the time of death, the mouth and operculum were open. When fish were exposed to fertilizers, the first dead fish was observed 5 h after exposure to 0.30 g/L

Table 1. Unionized ammonia and nitrite characteristics of water (n= 30 in 3 replicates)

Parameters	Ammonium sulphate concentrations (g/L)								
	0*	0.025	0.05	0.10	0.20	0.22	0.25	0.27	0.30
Unionized ammonia (ng/L)	39.1±0.17	63.2±0.44	98.4±0.65	152.5±0.77	198.3±0.56	245.1±0.83	281.4±0.81	348.6±0.62	394.5±0.51
Nitrite (μg/L)	29.3±0.33	36.1±0.56	48.3±0.78	60.4±0.71	64.0±0.39	71.3±0.84	86.4±0.89	108.5±0.37	130.3±0.93
	Calcium ammonium nitrate concentrations (g/L)								
	0*	0.15	0.20	0.25	0.30	0.35	0.40	0.50	
Unionized ammonia (ng/L)	45.15±0.29	51.88±0.74	76.24±0.61	114.95±0.55	158.87±0.78	225.66±0.80	271.59±0.69	231.86±0.78	
Nitrite (μg/L)	33.18±0.57	35.29±0.22	38.01±0.57	51.76±0.41	62.34±0.56	75.69±0.23	91.54±0.37	116.55±0.82	
	NPK-1 concentrations (g/L)								
	0*	0.012	0.50	0.10	0.25	0.40	0.55	0.70	
Unionized ammonia (ng/L)	43.15±0.56	48.80±0.11	53.30±0.34	59.43±0.56	63.33±0.67	67.41±0.27	71.28±0.81	73.23±0.95	
Nitrite (μg/L)	27.17±0.38	29.56±0.35	31.33±0.28	42.30±0.18	51.25±0.37	56.67±0.54	59.09±0.11	65.50±0.12	
	NPK-2 concentrations (g/L)								
	0*	0.05	0.20	0.35	0.50	0.65	0.80		
Unionized ammonia (ng/L)	40.15±0.45	46.44±0.43	51.32±0.51	55.67±0.69	57.86±0.78	59.19±0.63	62.70±0.37		
Nitrite (μg/L)	30.18±0.43	31.90±0.27	33.75±0.71	40.10±0.42	46.40±0.65	48.31±0.44	53.45±0.91		

*Control

or higher concentrations of ammonium sulphate and calcium ammonium nitrate while the first dead fish was observed 8 h after exposure to 0.55 g/L or higher concentrations of NPK-1 and NPK-2 (Figure 1). The toxicity of ammonium sulphate, calcium ammonium nitrate, NPK-1 and NPK-2 to rainbow trout increased with increasing fertilizer concentration and duration of exposure. The concentrations that killed 10% (LC_{10}) and 90% (LC_{90}) of fish varied with the fertilizers (Table 2). Ammonium sulphate was found to be more toxic to rainbow trout compared to composite fertilizers. The highest percentage of the mortality (60%) occurred during the first 48 h for ammonium sulphate. The mortality rate in fish increased significantly with increasing concentrations of fertilizers ($P<0.05$). Ammonium sulphate concentrations killing 50% of the rainbow trout were 0.242 g/L, 0.198 g/L, 0.165 g/L and 0.149 g/L during 24 h, 48 h, 72 h and 96 h, respectively (Table 2).

After exposing rainbow trout to different concentrations of fertilizers for 24 h, the concentrations lethal to 10% (LC_{10}) and 90% (LC_{90}) differed by a factor of 1.79, 1.32, 4.41 and 1.32 for ammonium sulphate, calcium ammonium nitrate, NPK-1 and NPK-2 (Table 2). The highest concentration of fertilizers caused the highest fish mortality. Significant differences were found in LC_{10-90} values obtained at different times of exposure ($P<0.05$). The time required to kill 50% (LT_{50}) of the rainbow trout to 0.20 g/L ammonium sulphate was 48 h 5 min (Table 3). In contrast, the LT_{50} value was 11 h 18 min for rainbow trout exposed to 0.30 g/L of ammonium sulphate. The time required to kill 50% (LT_{50}) of the rainbow trout at the highest concentrations of calcium ammonium nitrate, NPK-1 and NPK-2 were 12 h 23 min, 13 h 38 min and 17 h 47, respectively. As fertilizer concentrations were increased, LT_{50} values decreased ($P<0.05$).

The survived fish were transferred to flow through the tanks to observe fish behavior after the 96 h acute toxicity tests. Fish death continued one more day. The deaths were seen only 10% of the transferring fish. It was found that the fish freed the toxic effects of the fertilizers recovered more quickly.

Discussion

Behavioral responses of fish to most toxicants are the most sensitive indicators of potential toxic effects (EIFAC, 1983). The detection of abnormal activity is based on comparisons of the responses of exposed fish, either with activity measured during a baseline or pre-exposure period or observations of fish under a control treatment (Richmonds and Dutta, 1992). The changes in behavioral response started at 200 mg/L and above that concentration in the present study. The loss of equilibrium, erratic swimming, sudden swimming motion and excessive mucus are the main responses of fish to fertilizers. The responses recorded for the fish in this study are similar to those reported by other authors under various stress conditions (Paul and Banerjee, 1996; Rani *et al.*, 1997; Palanivelu *et al.*, 2005; Ufodike and Onusiriuka, 2008).

Nitrogen fertilizers can increase ammonium concentrations in the water (Kuma and Krishnamoorthi, 1983; Gangbazo *et al.*, 1995; Palanivelu *et al.*, 2005; Bobmanuel *et al.*, 2006). In water, ammonium exists in two forms, un-ionized ammonia (NH_3) and ammonium ion (NH_4^+). The equilibrium between the two forms of ammonium is controlled primarily by pH and temperature. These

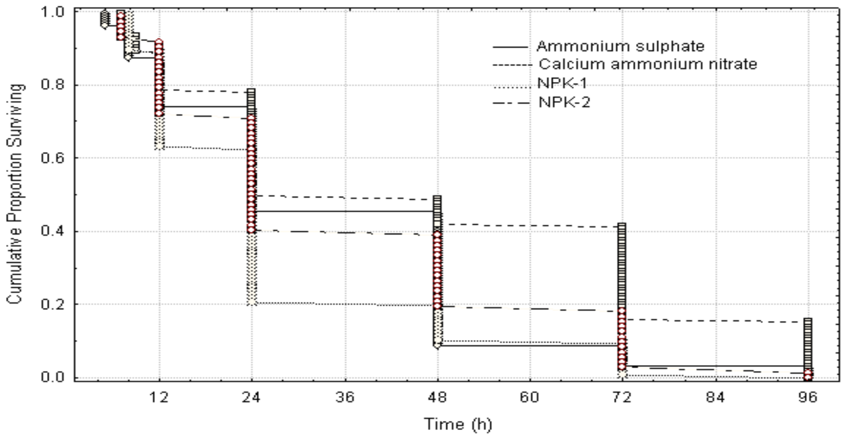

Figure 1. Cumulative survival (Kaplan-Meier) of rainbow trout after exposure to ammonium sulphate, calcium ammonium nitrate and composite fertilizers (NPK-1 and NPK-2) at different concentrations.

Table 2. Lethal concentrations (LC_{10-90}) of ammonium sulphate, calcium ammonium nitrate, NPK-1 and NPK-2 for rainbow trout (n= 30 in 3 replicates)

		Concentration (g/L) (95% confidence intervals)			
	Point	24 h	48 h	72 h	96 h
Ammonium sulphate	LC_{10}	0.173	0.151	0.123	0.115
		(0.137-0.193)	(0.126-0.172)	(0.092-0.142)	(0.090-0.133)
	LC_{50}	0.242	0.198	0.165	0.149
		(0.229-0.254)	(0.187-0.204)	(0.147-0.179)	(0.132-0.166)
	LC_{90}	0.311	0.214	0.206	0.184
		(0.292-0.344)	(0.208-0.238)	(0.193-0.223)	(0.167-0.207)
Calcium ammonium nitrate	LC_{10}	0.351	0.307	0.276	0.224
		(0.299-0.375))	(0.256-0.329)	(0.226-0.293)	(0.179-0.242)
	LC_{50}	0.408	0.352	0.307	0.259
		(0.384-0.446)	(0.331-0.372)	(0.289-0.327)	(0.240-0.277)
	LC_{90}	0.465	0.397	0.339	0.294
		(0.432-0.555)	(0.376-0.446)	(0.321-0.391)	(0.276-0.337)
NPK-1	LC_{10}	0.136	0.129	0.121	0.112
		(0.063-0.188)	(0.061-0.169)	(0.060-0.159)	(0.053-0.149)
	LC_{50}	0.368	0.306	0.263	0.258
		(0.326-0.413)	(0.272-0.343)	(0.231-0.297)	(0.227-0.291)
	LC_{90}	0.600	0.464	0.405	0.394
		(0.540-0.687)	(0.417-0.531)	(0.363-0.467)	(0.353-0.454)
NPK-2	LC_{10}	0.473	0.370	0.352	0.337
		(0.401-0.508)	(0.291-0.408)	(0.281-0.289)	(0.273-0.371)
	LC_{50}	0.549	0.442	0.425	0.408
		(0.515-588)	(0.403-0.476)	(0.388-0.462)	(0.375-0.447)
	LC_{90}	0.625	0.514	0.497	0.479
		(0.586-0.712)	(0.480-0.580)	(0.460-0.569)	(0.441-0.558)

speciation relationships are important in determining ammonium toxicity since un-ionized ammonia is generally more toxic to aquatic organisms than ammonium ion (In small amounts, ammonium causes stress and gill damage (Todgham *et al*., 2001; Wicks *et al*., 2002; Smart, 1978). In the present study, certain deformities and unusual swimming patterns were found in fish exposed to 200 mg/L and above concentrations. When we compared 96-h LC_{50} values among the four fertilizers, ammonium sulphate was the most toxic to rainbow trout while NPK-2 was less toxic. Because at the same concentration, un-ionized ammonia and nitrite concentration of ammonium sulphate fertilizer added water were higher than that of the other fertilizers. On the other hand, un-ionized ammonia and nitrite concentration of NPK-2 added water was less then the other fertilizers concentrations (Table 1).

The results of acute testing demonstrate that nitrogenous fertilizers exhibits acute toxicity to

Table 3. Lethal time (hours and minutes) (LT_{10-90}) of ammonium sulphate, calcium ammonium nitrate, NPK-1 and NPK-2 for rainbow trout (n= 30 in 3 replicates).

		Lethal time (95% confidence intervals)				
	Point	0.200 g/L	0.225 g/L	0.250 g/L	0.275 g/L	0.300 g/L
Aammonium sulphate	LT_{10}	12 h 15 m.	11 h 19 m.	9 h 1 m.	6 h 45 m.	6 h 30 m.
		(9 h 10 m.-20 h 1 m.)	(9 h 45 m.-16 h 18 m.)	(7 h 10 m.-15 h 9 m.)	(5 h 1 m.-6 h 50 m.)	(4 h 10 m.-9 h 30 m.)
	LT_{50}	48 h 5 m.	27 h 24 m.	22 h 24 m.	15 h 41 m.	11 h 18 m.
		(40 h 6 m.-55 h 35 m.)	(23 h 2 m.-32 h 39 m.)	(17 h 32 m.-26 h 34 m.)	(10 h 51 m.-20 h 48 m.)	(9 h 5 m.-16 30 m.)
	LT_{90}	86 h 34 m.	44 h 18 m.	34 h 52 m.	23 h 23 m.	16 h 54 m.
		(76h 13 m.-102h 12 m.)	(38 h 3 m.-56 h 24 m.)	(30 h 33 m.-43 h 22 m.)	(17 h 52 m.-28 h 24 m.)	(13 h 13 m.-20 h 40 m.)
		0.25 g/L	0.30 g/L	0.35 g/L	0.40 g/L	0.50 g/L
C. ammonium nitrate	LT_{10}	82 h 15 m.	53 h 19 m.	21 h 31 m.	13 h 45 m.	7 h 40 m.
		(61 h 53 m.-88 h 39 m.)	(43 h 19 m.-60 h 25 m.)	(18 h 10 m.-25 h 9 m.)	(9 h 12 m.-17 h 53 m.)	(5 h 30 m.-10 h 20 m.)
	LT_{50}	98 h 26 m.	70 h 50 m.	37 h 6 m.	18 h 13 m.	12 h 23 m.
		(93 h 5 m.-111 h 7 m.)	(65 h 28 m.-75 h 37 m.)	(29 h 2 m.-44 h 16 m.)	(14 h 44 m.-23 h 21 m.)	(9 h 45 m.-16 50 m.)
	LT_{90}	114 h 2 m.	87 h 30 m.	60 h 53 m.	37 h 13 m.	17 h 23 m.
		(105h 12 m.-155h 1 m.)	(81 h 58 m.-96 h 16 m.)	(51 h 44 m.-76 h 31 m.)	(31 h 52 m.-44 h 14 m.)	(13 h 55 m.-21 h 30 m.)
		0.10 g/L	0.25 g/L	0.40 g/L	0.55 g/L	0.70 g/L
NPK-1	LT_{10}	98 h 46 m.	69 h 25 m.	24 h 38 m.	9 h 55 m.	9 h 15 m.
		(91 h 43 m.-110 h 19 m.)	(51 h 33 m.-87 h 23 m.)	(19 h 29 m.-40 h 26 m.)	(7 h 13 m.-13 h 45 m.)	(7 h 25 m.-12 h 10 m.)
	LT_{50}	na	154 h 21 m.	39 h 54 m.	13 h 49 m.	13 h 38 m.
		-	(112 h 8 m.-251 h 31 m.)	(21 h 2 m.-54 h 43 m.)	(4 h 15 m.-18 h 37 m.)	(10 h 15 m.-16 41 m.)
	LT_{90}	na	223 h 40 m.	120 h 44 m.	33 h 38 m.	17 h 23 m.
		-	(180 h 38 m.-362 h 8 m.)	(91 h 4 m.-207 h 13 m.)	(27 h 15 m.-50 h 18 m.)	(14 h 45 m.-20 h 34 m.)
		0.20 g/L	0.35 g/L	0.50 g/L	0.65 g/L	0.80 g/L
NPK-2	LT_{10}	245 h 41 m.	111 h 50 m.	22 h 31 m.	14 h 22 m.	12 h 5 m.
		-	(98h 11 m.-150h 32 m.)	(15 h 5 m.-26 h 55 m.)	(10 h 17 m.-19 h 34 m.)	(7 h 8 m.-20 h 18 m.)
	LT_{50}	na	133 h 56 m.	36 h 16 m.	20 h 8 m.	17 h 47 m.
		-	(117h 18 m.-198h 41 m.)	(32 h 39 m.-41 h 21 m.)	(15 h 13 m.-26 h 33 m.)	(12 h 47 m.-23 34 m.)
	LT_{90}	na	na	50 h 42 m.	28 h 48 m.	21 h 9 m.
		-	-	(45 h 9 m.-59 h 31 m.)	(24 h 11 m.-32 h 14 m.)	(17 h 9 m.-28 h 43 m.)

na: not applicable

rainbow trout. Ufodike and Onusiriuka (2008) estimated the 96-h LC_{50} value of composite fertilizers for African catfish (*Clarias gariepinus*) ranged from 33.9 mg/L for Ca $(OH)_2$ to 1.25 g/L for $NaNO_3$. In another study, 96-h LC_{50} values of 28:0:0 and 10:34:0 for the rainbow trout were found to be 0.585 g/L and 1.342 g/L, respectively (Mac Kinlay and Buday, 1997). In the present study, the 96-h LC_{50} values for NPK-1 and NPK-2 were 0.258 g/L and 0.408 g/L, respectively. These results were higher than the values reported by Ufodike and Onusiriuka (2008) while smaller than Mac Kinlay and Buday (1997). The difference might be related to fertilizer composition, fish and physicochemical characteristics of the test water (Saha *et al*., 2002; Palanivelu *et al*., 2005).

Fertilizer might positively or negatively affect the ecosystem quality to the benefit or detriment of live aquatic organisms including fish (Yaro By *et al*., 2005). These effects for aquatic organisms moved away from toxic effects of pollutant may be deadly or recover quickly (Kuma and Krishnamoorthi, 1983; Yaro By *et al*., 2005). In the present study, all the deaths were only 10% after transferring 96 h toxicity test survivors to flow through tanks, which is similar with previous reports (Xu and Oldham, 1997; Little *et al*., 2002).

In summary, ammonium sulphate, calcium ammonium nitrate, NPK-1 and NPK-2 were toxic for rainbow trout. Compared to the fertilizers, the ammonium sulphate was more toxic to the rainbow trout while NPK-2 was less toxic. The toxicity increased with increasing of fertilizer concentration as well as exposure time. The results of the present study also indicate that the fish exposed to these fertilizers recover quickly when they were moved to freshwater. It is concluded that the fertilizers may have toxic potentials in the shallow water and therefore it should be carefully used in the areas closed to waterside.

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