Boletín de Biodiversidad de Chile 9: 1–10 (2014)
http://www.bbchile.com/

EFFECTS OF COPPER SULPHATE ON ZOOPLANKTON COMMUNITIES IN PONDS SUBMITTED TO AGRICULTURAL INTENSIFICATION

David León[1], Patricio Peñalver[1], Jesús Casas[2], Melchor Juan[2], Francisca Fuentes[2], Irene Gallego[2] and Julia Toja[1]

[1] *Department of Ecology and Plant Biology, University of Seville, Reina Mercedes Avenue, s/n cp 41012. Seville.*

[2] *Department of Plant Biology and Ecology, University of Almería. Email: leonmuez@us.es (corresponding author)*

Abstract

The effects of copper sulphate ($CuSO_4$) on zooplankton communities were investigated. Eight farm ponds at El Ejido, Almeria (South East of Spain) were sampled during an intensive and comparative monitoring study carried out between July 2008 and May 2009. This area, one of the most agriculturally intensive in the World, raises a great deal of controversy due to the impact of this type of management on natural wetlands and their associated biota. Copper sulphate is widely used in the area as an algaecide to clarify irrigation water, despite the majority of the water is extracted from the underground. According to the indications of the 8 estate owners, four of the sampled ponds were treated with CuSO4 at different concentrations, whereas the other four remained untreated. Microinvertebrate species richness and limnological parameters were measured in all ponds. Results confirm the negative effect of the algaecide over the microinvertebrate community.

Key words: Zooplankton, biocide, farm pond, agriculture, Andalusia, Spain

Efectos del sulfato de cobre sobre comunidades zooplanctónicas en charcos sometidos a intensificación agrícola

Resumen

Se ha estudiado el efecto del sulfato de cobre ($CuSO_4$) sobre las comunidades de zooplancton. Ocho balsas agrícolas en El Ejido, Almería (Sur de España) fueron muestreadas de forma intensiva y comparada entre Julio de 2008 y mayo de 2009. Esta zona, una de las más importantes áreas de agricultura intensiva del mundo, presenta una gran controversia debido al impacto que este tipo de manejo tiene sobre los humedales naturales y sus comunidades biológicas asociadas. El sulfato de cobre es ampliamente usado allí como alguicida para clarificar el agua de riego, aunque el origen de esta es mayoritariamente subterráneo. Según las indicaciones de los 8 propietarios, cuatro de las balsas fueron tratadas con diferentes concentraciones de $CuSO_4$, mientras que otras cuatro se mantuvieron sin el tratamiento. La riqueza de macroinvertebrados y diferentes parámetros limnológicos fueron medidos en todas las balsas. Los resultados confirman el efecto negativo del producto sobre la comunidad de macroinvertebrados estudiada.

Palabras clave: Zooplancton, biocida, balsa agrícola, Andalucía, España.

Recibido: 17 de Julio, 2013; Aceptado: 02 de Octubre, 2013, por Marcelo Rivadeneira

Introduction

It is well known that pesticides have a strong influence on the zooplankton (Rohr & Crumrine 2005; Relyea & Hoverman 2008). Moreover, several studies have reported a direct and indirect effect of herbicides (added to irrigation water to eliminate weeds from crops) on observed declines in animal biodiversity. For instance, Relyea & Jones (2009) investigated the effect of an herbicide on amphibian larvae; and de Noyelle *et al.* (1982) reported the effect of the herbicide atrazine on several planktonic crustaceans.

Copper Sulfate, also called Cupric Sulfate (CuSO4), is a chemical derivative of copper forming blue crystals, which are soluble in water and methanol. Apart from numerous industrial applications, this substance is used as an algaecide for water treatment. It is widely used in irrigation ponds, especially in those assigned to drip irrigation, as in Almeria, Spain, where treatment is applied to more than 75% of the pools.

In order to investigate the effect of this biocide on micro invertebrate communities, we carried out a field experiment in "Campo de Dalías", west of Almeria, Spain (Figure 1), an area intensively farmed where the use of copper sulphate is widespread and unregulated. Moreover, the area concentrates a huge density of agricultural ponds, which are responsible for the desiccation and degradation of the nearby wetlands of Punta Entinas-Sabinar and Albufera of Adra (Cruz-Pizarro *et al.* 2003; Pulido-Bosch *et al.* 2005). We selected two types of systems, some with naturalization and others artificial, and tested: - the colonisation rate of zooplankton in both systems; - the effect that the biocide may have on the community of micro-invertebrates.

Material and Methods

Farm ponds in the area were close to each other and presented similar characteristics; they were located on the banks of a shallow river, so water was of a similar quality (Figure 1). Type of construction (concrete), shape and size were also comparable, with a mean depth of 1.79 m (range 1.4 to 2.6) and a mean stored volume of 366 m^3 (range 214-758) (Figure 2).

Eight ponds were selected, drained and cleaned. Presence or absence of macrophytes was assessed, so that ponds that had previously held macrophytes (an indicator of naturalization) were not treated with copper sulphate (Group 1); whereas ponds from group 2 (previously without macrophytes) were treated with the biocide (Table 1). The pond "Vega 7", originally included into the group 1 (with macrophytes and without treatment) was eventually treated with the biocide some days after the beginning of the test (Table 2). This situation favored this pond was used as a control.

A total of 20 samples were obtained from each farm pond at: T0 (July 2008), T1, T5, T10, T17, T24, T31, T41, T51, T61, T71, T81, T91, T101, T131, T160, T190, T220, T250, T280 and T310 (May 2009). At each visit, samples of zooplankton, phytoplankton, periphyton and macroinvertebrates as well as in situ limnological parameters (Conductivity, Dissolved Oxygen, Secchi depth and Chlorophyll a) were measured. Macrophytes were identified and its % coverage estimated. Zooplankton samples were obtained with a tube of metacrylate of 10 cm of diameter and 2 meters of length. This was introduced in several places of the pond, in order to obtain an inte-

Table 1. Initial classification of selected farm ponds.
Tabla 1. Clasificación inicial de las balsas seleccionadas

GROUP 1: (WITHOUT TREATMENT)	Cortijo Rojas 2 (CR2)	Cortijo Rojas 1 (CR1)	**Vega 7 (VG7)**	Colegio (COL)
GROUP 2: (WITH TREATMENT)	Vega 9 (VG9)	Bellavista (BEL)	Higuera (HIG)	Cortijo Rojas 3 (CR3)

Table 2. Renewal time of water and concentration of $CuSO_4$ added for treatment of the selected ponds.
Tabla 2. Tiempo de renovación del agua y concentración de $CuSO_4$ añadida para las balsas seleccionadas

		Renewal time (days)	CuSO4 (mg/l Cu/yr)
group 1	**El Colegio**	19	0
	Ctjo. Rojas 1	35	0
	Ctjo. Rojas 2	38	0
group 2	**Vega 7**	15	1,9
	Higuera	24	1,8
	Vega 9	15	2,3
	Bellavista	45	2,5
	Ctjo. Rojas 3	23	3,3

Table 3. Macrophytes (genus) found in Group 1 ponds (% of covering).
Tabla 3. Macrófitas (géneros) aparecidas en balsas del grupo 1 (% de cobertura).

	January (approx T190)	February (approx T220)	March (approx T250)	April (approx T280)	May (approx T310)
CR1					
Chara	0	0	0	0	2
Zannichellia	37	64	67	72	61
CR2					
Chara	12	18	24	30	34
Potamogeton	0	0	1	1	2
COL					
Chara	0	4	6	9	14

grated sample of 20 liters. These were filtered by a 45 μ of pore diameter. Every sample was conserved in formaldehyde (4%) until the identification with microscope, in the laboratory. Differences in species richness between group 1 and 2 were tested by the non-parametric statistic U-Mann Whitney; software used was SPSS v15.0.

Figure 1. Area of study and location of farm ponds (Google Earth)

Figura 1. Área de estudio y localización de las balsas (Fuente: Google Earth)

Results

First macrophytes appeared in January (T190), six months after the beginning of the experiment (Table 3). *Zannichellia* spp. *Chara* sp. and *Potamogeton* sp. colonized CR 1, CR 2 and COL, the three ponds not treated with the herbicide. VG 7, which belonged to Group 1 (naturalised ponds) but was finally treated, did not hold macrophytes in the 11 months of the experiment.

Table 4. Mean and total taxa (accumulated), by pond, along the 11 months of study.

Tabla 4. Promedio y número total de taxones (acumulado) por balsa, en los 11 meses de estudio.

		Mean	**Total**
Group 1	COL	5,1	24
	CR1	7,8	24
	CR2	4,3	20
	VG7	1,1	5
Group 2	HIG	3,5	13
	VG9	2,8	17
	BEL	0,9	7
	CR3	1,4	9

Richness of micro invertebrates showed differences between ponds, especially among those with biocide treatment (BEL, HIG, VG9, CR3 and VG7) and those without it (CR2, COL and CR1). Both mean and accumulated richness values were higher on ponds from Group 1 (without treatment) than those in Group 2 (Table 4). Results from the nonparametric test MW over mean values of richness by group of ponds and over the total number of registered species confirmed that there were significant differences between the two groups of ponds ($p < 0.05$).

It is remarkable the similarity on water conditions among 8 farm ponds (Conductivity, Dissolved oxygen and planktonic chlorophyll Table 5).

Discussion

The results showed in this study suggest that cooper sulphate has a clear influence over biologic communities, so use of it should be regulated, at least in areas with natural wetlands on its surroundings. Micro invertebrate richness was higher in ponds from Group 1, even though all ponds presented similar values of chlorophyll, dissolved oxygen, conductivity and Secchi (Table 5).

Table 5. Values of planktonic Chlorophyll (mg/l), dissolved oxygen (mg/l) and conductivity (µS/cm) during the 8 months of study.

Tabla 5. Valores de clorofila planctónica (mg/l) oxígeno disuelto (mg/l) y conductividad (µS/cm) durante los 8 meses de estudio.

	El Colegio			**Ctjo. Rojas 1**			**Ctjo. Rojas 2**			**Vega 7**			**Higuera**			**Vega 9**			**Bellavista**			**Ctjo. Rojas 3**		
	Cl	**%DO**	**C**	**Cl**	**%DO**	**C**	**Cl**	**%DO**	**C**	**Cl**	**%DO**	**Cond**	**Cl**	**%DO**	**C**	**Cl**	**%DO**	**C**	**Cl**	**%DO**	**C**	**Cl**	**%DO**	**C**
T0	-	-	-	4	-	4074	2	-	1229	8	-	3014	0	-	-	1	-	2747	-	-	-	-	-	-
T1	0	102	2120	0	102,1	1260	4	101,1	1250	1	55,4	4500	3	110	1266	1	97,8	1443	0	97,8	1250	0	-	-
T5	16	105	2370	7	171,3	1275	6	112,3	1258	44	118	4595	1	130	1269	1	94	3914	0	94	1235	0	88,8	1222
T10	4	109,8	3820	2	125	1290	4	124,8	1226	28	126	4660	1	145	1250	0	90	1273	0	90	1302	2	92,1	1310
T17	13	150,1	3960	1	115	4126	1	137	1206	41	148	4550	1	112,5	1209	0	84	1198	0	91	1190	1	95,2	1420
T24	8	133,3	3820	2	115	4010	5	129,5	1295	15	128	4550	1	129,2	1234	1	100,4	1206	0	102	3918	1	99,2	2760
T31	2	107,4	3650	6	112,4	3960	3	112,5	1451	12	101	4480	1	114,7	1243	3	101	1300	0	100	4000	10	91,5	4350
T41	1	125,8	2660	2	135,6	4030	4	121,3	1516	23	148	4380	1	109,2	4000	1	102	3900	0	96	4200	10	129,8	4640
T51	5	100	2980	1	133,7	4170	247	231,4	3910	5	118	4360	7	112	4270	1	103,3	4720	3	92,5	4270	8	107,5	4820
T61	12	121,4	3020	2	157	4170	4	88,4	3620	21	157	4378	4	123	4340	11	129,4	4380	6	127,8	4140	1	102,7	4640
T71	4	100,4	2970	0	158,5	4160	4	84,5	4180	3	111	4335	64	149,2	4170	8	126,7	3960	7	125	3840	3	104,8	4580
T81	3	103	2949	0	133	4170	12	128,7	3890	17	88,5	2965	16	129,5	4050	3	107,9	4810	4	109,1	3860	3	104,5	4510
T91	1	101	3504	0	139,9	4090	5	112,3	3910	62	130	2853	8	111,6	4100	2	109,9	4230	8	110	3260	2	105	4520
T101	4	96	1805	0	209	4166	5	137,8	3680	23	112	2912	20	104	4120	1	110,5	4190	8	115,2	3170	1	106	4419
T131	2	93,2	2030	0	118,5	3186	1	120,2	3890	120	144	2661	0	86,7	3477	1	110	3912	3	91,6	2704	3	87,3	3635
T160	1	109	1974	0	90,5	2922	4	107	2438	4	123	2891	0	91,6	3395	105	88	2661	1	83	4966	3	102,2	3261
T190	3	125	2625	0	135	3153	12	103	2640	9	121	3215	1	103	3150	2	106,2	2347	1	102,8	5505	4	150,6	3332
T220	1	190	2965	1	185	3395	3	144,8	2946	21	123	3565	0	111,5	3264	1	111,7	2609	2	131,8	7066	5	146	3578
T250	0	168	3147	1	125,5	3759	3	175,7	3466	19	175	4240	1	116	3461	1	107,6	3347	1	98	7720	13	118	3735
T280	4	158,3	3510	2	300	3650	1	237	3415	-	-	-	0	148	3581	1	119,4	3719	3	126	7650	7	164,9	4430
T310	-	-	-	-	-	-	1	169,4	3760	-	-	-	1	122,6	3770	2	142,4	4300	11	120,7	7480	-	-	-

Figure 2. Distribution and appearance of ponds.
Figura 2. Distribución y aspecto de las balsas.

Table 6. Presence and absence of different taxa in the ponds and point(s) on time at which they were identified. Dates are from T0 (July 2008) to T310 (May 2009).
Tabla 6. Presencia/ausencia de los diferentes taxones en las balsas y momento en el que fueron identificados. Tiempos entre T0 (Julio 2008) y T310 (mayo 2009).

Taxa	CR2	COL	CR1	BEL	HIG	VE9	CR3	VE7
ROTIFERA								
Asplanchna sp.	T71	T81	T41					
Brachionus calyciflorus	T51-T71	T81	T41					T24-T92
Brachionus plicatilis	T24-T71	T17,T42,T81-T91	T5-T53		T0-T101	T1-T101	T238	T24-T30
Euchlanis dilatata						T41		
Euchlanis incisa	T51							
Euchlanis sp		T118-T168	T31			T1		
Hexarthra bulgarica	T5-T71	T1-T81	T5-T81, T237	T72-T82	T1, T24-T81, T101, T182, T207-T282	T41, T73--T92, T158, T233-T283		
Keratella quadrata		T218	T137					
Lecane luna	T292	T31, T218	T53-T82	T131	T31-T41	T92-T131	T263	
Lecane lunaris	T31, T61, T242-T292	T101	T31-T81		T1, T17-T31, T131			
Lepadella patella	T24-T31, T242	T10, T91	T10-T61	T1	T31, T72-T81	T1	T263	
Polyarthra dolicoptera	T31, T90-T131	T17-T218	T17-T81			T283		
Tichocerca similis	T17-T71, T131, T217, T267	T17-T101, T218	T17-T72, T101		T0-T131, T232, T282	T173-T283	T31-T263	T24, T92, T132-T157,T207
COPEPODA								
Nauplii ciclopidae	T5-T101, T160, T267	T10-T42, T71-T118, T193-T218	T5-T237	T72-T91	T81.T101, T151-T182, T232,T282	T81-T101, T158-T183, T233-T283	T73-T81, T213-T273	T1, T207
Nauplii diaptomidae	T10, T24		T81	T72-T82	T182, T282	T73		
Ciclopidae								
Acanthocyclops americanus	T10-T31, T61-T101, T160, T267	T10.T17, T31-T42, T61-T81, T101, T168, T218	T10-T41, T72-T137, T187, T237	T72-T131	T91-T131, T257	T92, T258	T41, T73, T238	T1, T182
Acanthocyclops robustus	T17	T17, T61	T17, T41, T212	T91-T131	T257		T238	
Cyclops abyssorum			T212-T237					
Tropocyclops prasinus						T1		
CLADOCERA								
Alonella nana	T192-T217		T137-T212					
Ceriodaphnia quadrangula						T92		
Daphnia magna	T17				T232-T282			
Macrothrix hirsuticornis			T81-T137					
Moina micrura			T41					
Pleuroxus aduncus		T168-T218	T101, T187					
Pleuroxus letournexi	T217-TT242	T71,T91-T118, T168-T218	T91-T237					
Simocephalus vetulus	T160	T24-T42, T71, T91, T193-T218	T5-T237		T131-T282	T1, T101-T208	T41, T238	
OSTRACODA								
ostracod sp.1	T0-T5, T24, T51, T217, T292	T10, T24, T168-T218	T5, T17.T41, T91-T212			T5		
ostracod sp.2		T91-T101	T5, T17-T41, T72-T81, T101-T137, T187-T212			T1, T73,T92, T233		

Differences in richness could be due, in part, to the positive effect that macrophytes have on periphyton (which is used as a trophy resource by sessile rotifers and chydorids); this was limited in farm ponds of Group 2, because of the detrimental effect of copper sulphate on macrophytes. In farm ponds without treatment there was not an asymptote in the accumulated richness, for eleven months. However, in ponds with the treatment, accumulated number of species reached a stable value very soon. Therefore, there seems to be a clear relationship between addition of copper sulphate and the lowest values of richness. Predation related difference between ponds, were discarded due to the absence of fish or other predators.

In conclusion, it seems to be an effect of the biocide on micro invertebrate community, direct or indirect; this suggestion has been detected by other authors on similar studies (De Noyelles *et al*., 1982; Rohr & Crumrine, 2005; Relyea & Diecks, 2008; Relyea, 2009; Brandl, 2005). This study provides another evidence of the detrimental effect of copper sulphate not only on primary producers, but also −directly or indirectly− on zooplankton.

Acknowledgements

This study was supported by The "Consejería de Medio Ambiente de la Junta de Andalucía", through a Project for the characterisation of farm ponds in Andalusia, South of Spain.

We want to thank to the farm pond owners for their help towards the development of this study. We also especially thank to Estefanía Pérez for her suggestions on the English version and to reviewers and editor from the "Boletín de Biodiversidad de Chile" for their open and constructive comments.

Referencias bibliográficas

Brandl, Z., 2005. Freshwater copepods and rotifers: predators and their prey. *Hydrobiologia*, 546: 475–489.

Cruz-Pizarro, L., I. De Vicente, E. Moreno-Ostos, V. Amores & K. El Mabrouki, 2003. Estudios de diagnóstico y viabilidad en el control de la eutrofización de las lagunas de la albufera de adra. *Limnetica*, 22(1-2): 135–154.

De Noyelles, F., W. Dean Kettle, D. E. Sinn, 1982. The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticida in the United States. *Ecology*, 63(5): 1285–1293.

Pulido-Bosch, A., F. Sánchez-Martos, J. L.Martínez-Vidal & F. Navarrete, 2005. Groundwater problems in a semiarid area (low Andarax river, Almería, Spain). *Environmental Geology*, 20: 195–204.

Relyea, R. & N. Diecks, 2008. An unforeseen chain of events: lethal effects of pesticides on frogs at sublethal concentrations. *Ecological Applications*, 18(7): 1728–1742.

Relyea, R., 2009. A cocktail of contaminats: how mixtures of pesticides at low concentrations affect aquatic communities. *Oecologia*, 159: 363–376.

Relyea, R. & J. T. Hoverman, 2008. Interactive effects of predators and a pesticide on aquatic communities. *Oikos*, 117: 1647–1658.

Relyea, R. & D. K. Jones, 2009. The toxicity of roundup original max to 13 species of larval amphibians. *Environmental Toxicology and Chemistry*, 28(9): 2004–2008.

Rohr, J. R. & P. Crumrine, 2005. Effects of an herbicide and an insecticide on pond community structure and processes. *Ecological Applications*, 15: 1135–1147.