BERLIN 6th SETAC World Congress 2012

INTEGRATIVE APPROACH FOR THE RISK ASSESSMENT OF FRESHWATER RESERVOIRS INFLUENCED BY INTENSIVE AGRICULTURAL ACTIVITIES: A CASE-STUDY OF ALQUEVA RESERVOIR (SOUTH OF PORTUGAL)

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INTRODUCTION

Freshwater reservoirs located in intensive agricultural areas are more vulnerable to chemical "stressors", such as pesticides. So, the characterization of these water bodies for prevalent pesticides is extremely important, once most of these compounds are used in an indiscriminate way by farmers and induce toxic effects in species of aquatic ecosystems and benthic communities.

The Alqueva reservoir, located at Guadiana's basin (South of Iberian Peninsula, Portugal), constitutes an essential water resource for multiple regional uses, such as: reinforcement of water supply to the populations (drinking water), irrigation, and generation of electric energy. The influence of the Mediterranean climate, with high temperatures and low precipitation, together with the fact of excessive inputs of nutrients and intensive farming practices, induces a strong variability in water quality of this reservoir. Previous studies showed that Alqueva's water quality and its ecosystem are influenced by the presence of several pesticides and its metabolites, such as atrazina, endosulfan sulphate, chlorpyriphos, diuron, diazinon and metolachlor (Palma *et al*., 2009).

The PURPOSES of this study were:
(i) to evaluate the dynamic in surface water and sediments of Alqueva reservoir, of twenty three pesticides, which belong to the classes of Triazines - deisopropylatrazine, desethylatrazine, simazine, cyanazine, atrazine, and terbutylazine; Phenylureas - chlortoluron, isoproturon, diuron, and linuron; Organophosphates - dimethoate, diazinon, fenitrothion, malathion; Anilines - metolachlor, alachlor, chlorfenvinphos (CFP) and propanil; Thiocarbamates - Molinate; Acidics - Mecoprop, 2,4-Dichlorophenoxyacetic acid (2.4-D), Bentazone, 2-methyl-4-chlorophenoxyacetic acid (MCPA);
(ii) to correlate the pesticides concentrations measured with the toxicity values obtained in the both matrices (water and sediments) of Alqueva reservoir.

MATERIALS & METHODS

- **WATER AND SEDIMENTS SAMPLES** were collected during 2011, at each of the five sampling sites selected along Alqueva reservoir: upstream ((Ajuda (Aj), Alcarrache (Ac), Álamos (Al)), and at the middle ((Mourão (Mr), Lucefecit (Lf)) of the reservoir.

- **WATER AND SEDIMENTS PHYSICOCHEMICAL CHARACTERIZATION**: # **water** - pH, temperature, dissolved oxygen, conductivity, chloride, phosphorus, Kjeldahl nitrogen, biochemical oxygen demand and chemical oxygen demand; ## **sediments** – pH, conductivity, organic matter, nitrogen, phosphorus, metal micronutrients.

- **PESTICIDES** extraction in water was based on the automated on-line solid phase extraction (on-line SPE) procedure described by Kampioti *et al*. (2005), while extraction in sediment was carried out by pressurized liquid extraction (PLE). In both cases, the analysis was based on liquid chromatography-tandem mass spectrometry (LC-MS/MS). Water samples were taken in February, April, June, July and September and (ii) sediments in February, April and June.
- **ECOTOXICITY CHARACTERIZATION OF SURFACE WATER AND SEDIMENTS** using a battery of acute bioassays: luminescent inhibition of *Vibrio fischeri* (ISO 11348-2, 1998), *Daphnia magna* immobilization (ISO 6341, 1996) and *Thamnocephalus platyurus* mortality (Persoone, 1999).

RESULTS & DISCUSSION

▪According to the recommended levels allowed by the Portuguese Legislation concerning the quality of surface water for multiple uses (Decreto-Lei nº 236/98), pH, temperature, EC and DO values from water samples were within the limits (**Table 1**).

Table 1 - Range of main physicochemical parameters, recorded in water samples, during 2011.

Sampling Stations	pH	Temperature (ºC)	EC (µS cm^{-1})	DO (%)
Álamos	7.1-8.6	13.0-25.0	325-369	90-132
Alcarrache	7,7-8,9	14.0-25.0	275-351	93-144
Mourão	6.8-8.6	12.5-25.7	296-409	98-104
Lucefécit	7.2-8.1	9.8-25.1	472-493	91-146
Ajuda	7.6-8.1	9.8-24.0	451-493	94-119

▪The **Figure 1** showed the patterns of organic descriptors (CBO_5, CQO) and nutrients (Total phosporus, Kjeldahl nitrogen) during 2011, from Alqueva Reservoir.

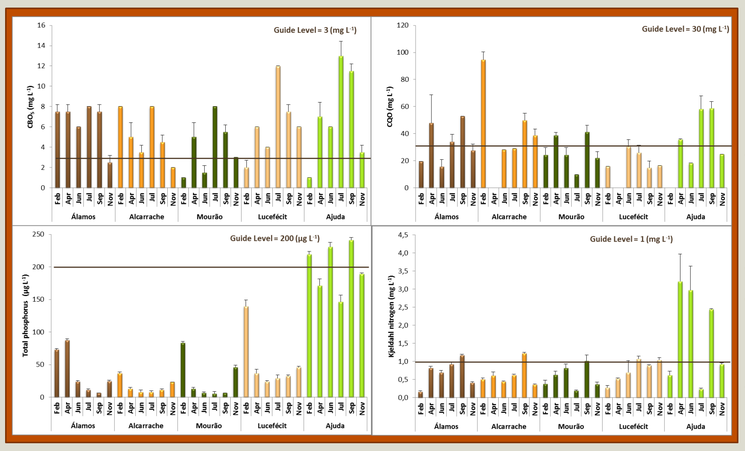

Figure 1 – Seasonal variations of some chemical parameters from the sampling stations of Alqueva reservoir, during 2011

▪The CBO_5 exceeded the guide level at most of the months, in all sampling stations. The spatial variation indicated that water samples from Ajuda and Lucefécit had the greater levels of this parameter.
▪The CQO values ranged from 9.0 and 94.0 mg O_2 L^{-1} and were higher at wet period. Alcarrache and Ajuda were the sites with greater CQO contents.
▪With regard to nutrients, Ajuda was the only station that showed values of nitrogen and phosphorus values which exceeded the guidelines in most of the months.

▪Of the **25 pesticides** analysis in the water samples, 21 were found to be present in some or all the samples analysed. The four undetected compounds were cyanazine, fenitrithion, fenitrothion oxon and malathion. The most abundant pesticides were bentazone (4172.26 ng L^{-1}), terbuthylazine (3451.3 ng L^{-1}), MCPA (1262.46 ng L^{-1}), 2,4-D (1046.60 ng L^{-1}) and metolachlor (1026.29 ng L^{-1}) **(Figure 2).**
▪The highest levels of bentazone were observed at Ajuda (1769.02 ng L^{-1}) in July 2011.

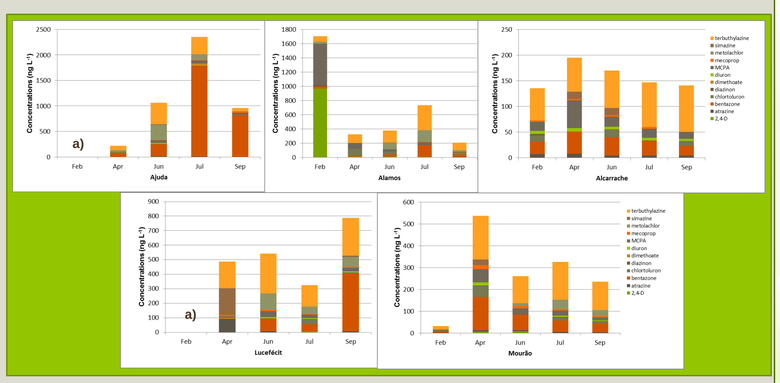

Figure 2 – Spatial and seasonal variation of **terbutylazine, simazine, metolachlor, mecoprop, MCPA, diuron, dimethoate, diazinon, chlortoluron, bentazone, atrazine and 2.4-D, during the months of February (Feb), April (Apr), June (Jun), July (Jul) and September (Sep) of 2011.** a) pesticides not determined.

▪Terbuthylazine maximum concentrations were quantified, in June, also at Ajuda (422,81 ng L^{-1}), sampling station located in the north of the reservoir, near Spain.
▪2,4-D has reached the highest concentration at Álamos in February.
▪The areas most polluted by pesticides were Ajuda, Lucefecit and Álamos, located in the northern and in the middle of the reservoir, respectively.
▪Considering the seasonal variation, the patterns showed that the highest contents of pesticides were found at the dry period, with the exception to Alamos. At this location, February was the month when there was the main concentration of total pesticides, exceeding the guide level (1000 ng L^{-1}) of the Portuguese Legislation (Decreto-Lei nº 236/98).

RESULTS & DISCUSSION

Table 2 – Range of pH, electrical conductivity (EC) organic matter (OM), nitrogen ($N_{kjeldahl}$) and pesticides, in the different sediments collected at Alqueva reservoir

		Al	Ac	Mr	Lf	Aj
$pH_{elutriate}$		7.05-7.43	7.03-7.28	7.34-7.78	7.49-7.76	7.82-8.20
$CE_{elutriate}$(ms/cm)		547-678	423-737	803-928	662-719	410-926
OM(%)		7.63-20.30	2.54-14.21	2.26-11.61	11.33-23.38	0.90-8.39
$N_{kjeldahl}$ (%)		0.21-0.47	0.04-0.82	0.18-0.36	0.27-0.75	0.04-0.30
Pesticides (ng L^{-1})	CFP	< L_{det}	< L_{det}-0.63	< L_{det}-1.43	< L_{det}-0.93	< L_{det}-0.98
	Chlortoluron	< L_{det}-0.99	0.31-2.53	< L_{det}-0.67	< L_{det}-0.93	< L_{det}-0.88
	Diuron	< L_{det}-3.28	1.69-3.18	< L_{det}-0.95	1.33-2.17	0.69-1.64
	Propanil	< L_{det}-0.44	< L_{det}	< L_{det}	< L_{det}	< L_{det}-0.40
	Terbuthylazine	< L_{det}-0.29	< L_{det}-0.25	1.01-1.07	0.48-0.88	0.55-0.83

L_{det} (detection limit); L_{det} CFP (0.51 ng L^{-1}) ; L_{det} Chlortoluron (0.30 ng L^{-1}); L_{det} Diuron (0.20 ng L^{-1}); L_{det}Propanil (0.38 ng L^{-1}); L_{det} Terbuthylazine (0.13 ng L^{-1}).

▪ In general, Alqueva sediments were characterized by values of pH near to the neutrality and great levels of organic matter. The worst case was Ajuda with the highest values of pH and conductivity and poor contents of nutrients and organic matter.

▪The amounts of pesticides in sediments were low, of the 23 pesticides analyzed only 5 were detected in the sediments. Diuron was the pesticide that was detected in large quantity followed by chlortoluron.
▪Diuron and CFP were detected in sediments, always, in concentration below their EQS values.
▪Alcarrache and Lucefécit were the areas with more pesticides amounts.

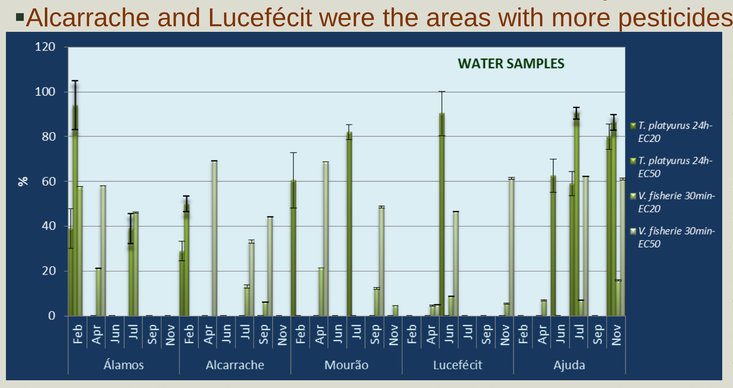

Figure 3 – Toxic effect (%) induced by the water samples during the study. **24h-$EC_{20,50}$(%)** values calculated for *T. platyurus* bioassay after 24 h of exposure to water samples (mean ± SD; n=4). ASTM was used as control. **30min- $EC_{20,50}$(%)** values calculated with luminescent bacteria bioassay after 30 min exposure to water samples. NaCl 2% was used as control (mean ± SD; n=2).

▪Generally water samples induce low toxicity to the species used in the study **(Figure 3).**
▪Despite this fact, the results showed that *V. fisheri* was the most sensitive followed by *T. platyurus* and finally by *D. magna* (specie unresponsive to the samples from this reservoir).
▪Regarding the spatial dynamic of toxicity, we can observe that Ajuda was the site that induced more toxic responses, followed by Álamos and Lucefécit.

▪ In the main, elutriates samples were more toxicity than water samples **(Figure 4).**
▪As for water samples, the *V. fisheri* was the most sensitive specie.
▪Although, the crustacean *D. magna* was not sensitive to water samples, some elutriates promoted a few toxicity to this specie (48h-$EC_{20,50}$ between 14 and 94%), these results were in agreement with a previous study (Palma et al., 2010a) .
▪Sediments with higher toxicity were from Ajuda and Mourão.

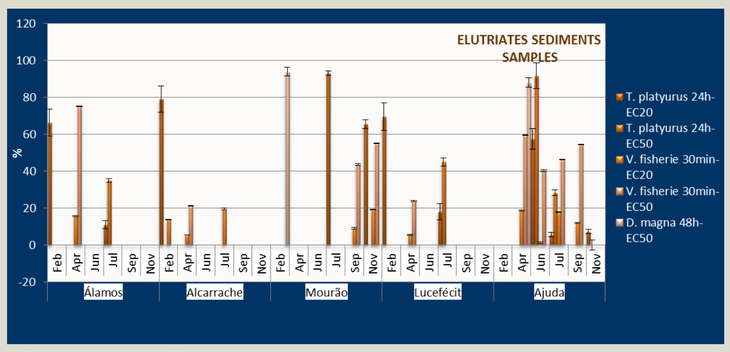

Figure 4 – Toxic effect (%) induced by the sediments elutriates during the study. **24h-$EC_{20,50}$(%)** values calculated for *T. platyurus* bioassay after 24h of exposition (mean ± SD; n=4). ASTM was used as control. **30min- $EC_{20,50}$(%)** values calculated with luminescent bacteria bioassay after 30 min of contact. NaCl 2% was used as control (mean ± SD; n=2).

CONCLUSIONS

•The water quality is greatly influenced by the organic contents, tributary to the reservoir, as we already had observed in a previous study performed at the reservoir (Palma et al., 2010b).
•In general the levels of nutrients were lower than the values obtained during 2006/2007. At the present only Ajuda surpassed the guide values .
• Comparing the pesticides results obtained, in the current work, with those reported in a previous one conducted in the same area in 2006/2007 (Palma et al., 2009) the concentration of total pesticides decreased and there was a change in patterns of pesticides quantified. These facts can be explained both by changes in agricultural practices and by the low rainfall that characterized the region.
•The major classes identified were acidics and triazines.
•The spatial evaluation showed that the northern area of the Alqueva reservoir (Ajuda, Lucefécit and Alamos) is the most affected by pesticide contamination, which is consistent with the toxicological profile obtained in the water samples.
•The toxicity induced by the sediments can not be explained only by the amounts of pesticide obtained, but by the joint action with other contaminants such as toxic metals, which in this study surpass the levels of Freshwater Guidelines Quality for the protection of aquatic life.

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The authors would like to thank FCT and COMPETE for financial support through the PTDC/AAC-AMB/103547/2008

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