Rev. Bras. Saúde Prod. An., v.8, n.3, p. 217-228 jul/set, 2007 **http://www.rbspa.ufba.br**
ISSN 1519 9940

Daphnia magna – bio-indicator of pollution from poultry and pig abattoir effluents

Uso de "Daphnia magna" como bioindicador da poluição gerada por efluentes de abatedouros de aves e suínos

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SUMMARY

The current research investigated the use of *Daphnia magna* in foreseeing environmental risks from poultry and pig abattoir water effluents during the rainy and dry seasons, in the state of São Paulo, Brazil. Three effluents from poultry abattoirs and two effluents from pig abattoirs had a potential impact on treated industrial effluents discharged in Class II and III water bodies.

Key-words: bioassays, environmental impact, metals, toxicity, water, wastewater.

RESUMO

Este estudo foi efetuado objetivando obter informações sobre a utilização da *Daphnia magna* na predição dos riscos ambientais das águas e dos efluentes de abatedouros suinícolas e avícolas, nos períodos de chuva e de seca. Verificou-se que dentre os efluentes industriais, tratados e lançados em corpos d'água de Classes II e III, três efluentes de abatedouros avícolas e dois de suínos, apresentaram potencial impactante.

Palavras-chave: água, água residuária, bioensaio, impacto ambiental, metais, toxicidade.

INTRODUCTION

Cleanliness and hygienization of premises, apparatuses and tools in industries dealing with animal products are extremely important to avoid food-borne diseases, food contamination and, as a consequence, disease transmission, with different types of impact on people's health. Babbitt et al. (1973) and Andrade & Martyn (1982) reported that most synthetic petrochemical detergents used in cleanliness and hygiene are not successfully removed by the industries' treatment of effluents. Furthermore, they are not degradable by the microbiota usually found in rivers, lakes and sea.

Heavy metals, such as zinc and copper, are fundamental for animal feeding

since they enhance growth and prevent diseases. These metals are also essential factors for all life systems and crucial to many biochemical cell activities (Yang et al., 1999). Although the above-mentioned metals are important to animal physiology, they may be potentially toxic to aquatic organisms, due to the fact that they deplete lakes of dissolved oxygen and inhibit the capacity of the water to reduce microbial charges and self-cleansing (Abbasi et al., 1988; Yang et al., 1999; Villegas-Navarro et al., 2001).

Bioassays (Bitton, 1983; Bervoets et al., 1996; Caracterização, 2000) may be an alternative and complementary tool used in the place of the traditional chemical analyses to determine toxicity of environmental samplings. Daphnia magna is a fresh water zooplankton species, highly sensitive to the environment's physical and chemical variations. Since the micro-crustaceans have been used in assays involving acute and chronic toxicity, pure and complex chemical elements may be detected in industrial outputs (Villegas-Navarro et al., 1997; Emmanuel et al., 2004).

Daphnia magna, accepted in toxicology in several countries, including France and the United States, has been used to monitor residuary waters, and thus recommended as an appropriate investigatory method to determine tolerated concentrations of pollutants and to establish quality criteria for water discharged from effluents (Vasseur et al., 1984; Villegas-Navarro et al., 1997; Sanchez et al., 1998; Villegas-Navarro et al., 2001; Emmanuel et al., 2004).

The Environmental Sanitation Technology Company (CETESB) in the state of São Paulo, Brazil, has conducted toxicity tests to control liquid effluents (CETESB, 1990) and bioassays have been employed as a complement to chemical analysis (CETESB, 1990). In fact, toxicity tests or bioassays have been efficient alternatives for prevention and correction methods in water pollution control by toxic chemical compounds.

Current research diagnosed residual waters in poultry and pig abattoirs during dry and rainy seasons, in the state of São Paulo, Brazil. Bioassays with Daphnia magna were undertaken at the effluents of both abattoirs' treatment systems.

MATERIALS AND METHODS

Survey of abattoirs, characterization of sampling sites, collection and transport of samples

Seven poultry and seven pig abattoirs from the state of São Paulo, Brazil, were evaluated. Whereas four poultry abattoirs were inspected by the State Inspection Service (SISP) and three by the Federal Inspection Service (SIF), four pig industries were inspected by the SIF and three by SISP.

Questionnaires were distributed during technical visits to the abattoirs. Tables 3 and 4 show details on poultry and pig abattoirs, respectively.

Water effluent samples (20 mL) from both abattoirs were collected from each sampling site. At the lab, samples were kept at 4°C, from 36h to 14 days (CETESB, 1991; ABNT, 1993; APHA et al., 1998), till the start of acute and chronic toxicity tests with Daphnia magna.

Samplings were carried out on working days, in the morning, from May to September 2003 (dry period) and from January to March 2004 (rainy period). Metric tapes were used to determine sites 100m upstream and 100m

downstream the emission point of the effluents.
Samples were transported in isothermal boxes, with ice, to the Biomass Laboratory of the Rural Engineering Department, to the Ecotoxicology of Agrotoxics and Occupational Health Lab of the Phytosanitary Department, both administered by the UNESP-FCAV.

Laboratorial analyses

Culture of *Daphnia magna*

Daphnia magna was originally obtained from lab cultures of the Ecotoxicology of Agrotoxics and Occupational Health Laboratory of the Ecotoxicity Department of the Faculty of Agrarian and Veterinary Sciences, UNESP, Jaboticabal SP Brazil. All stages in culture maintenance followed ISO (1982), CETESB (1991), ABNT (1993).
Cultures were incubated at 20 (20 ± 2 °C, under 1,000 lux light intensity, 16h-8h light-dark photoperiod.
Organisms were cultivated in 2.000 mL-crystallizers with basal medium M4 (DIN 38409, 1989; DIN 38412, 1989) and fed on alga Scenedesmus subspicatus (3 x 10^6 / organisms / day). Changes of culture medium were done twice a week, prior to feeding.

Acute toxicity tests *with Daphnia magna*

Acute toxicity tests followed instructions by DIN 38409 (1989), DIN 38412 (1989), CETESB (1986), CETESB (1991), ABNT (1987) and ABNT (1993), and were divided into a preliminary and a definitive stage.
The separation of neonates, 6-24h, constituted the first stage in acute toxicity testing. Solutions for tests (concentrations of samples, in percentages, of 100; 50; 33.33; 25; 16.66; 12.5; 8.33; 6.25; 4.16; 3.125) were obtained directly from sample of effluent water and/or by dilutions.
Experimental lots of the preliminary stage were composed of glass test tubes with 5 neonates in each tube and 10mL of test solution (total volume), exposed 48h at 20 ± 2 °C, in the dark, and without feed. Control lot consisted of culture medium with the same number of organisms. At the end of the period immovable neonates in each tube were counted while temperature (20 ± 2 °C), pH (7.0 – 7.9) and dissolved oxygen (8.3 – 9.1 $mg.L^{-1}$) were registered by U10 water analysis multi-parameter probe (HORIBA, 1991).
According to the readings, this preliminary phase indicated lethal concentration intervals for the definitive assay. The lowest concentration, which caused immobility to all organisms, had the highest concentration of test solutions; the highest concentration in which immobility in neonates was not extant had the lowest concentrations of test solutions.
Definitive stage was taken from concentration rates established in the preliminary test, by a series of intermediary concentrations (dilutions) (concentrations of 25; 20; 16.66; 12.5; 10; 8.33; 7.14; 6.25; 4.54; 4.16), with four repetitions. Twenty organisms were used for each dilution (5 organisms in each of the 4 repetitions, with 10mL test solution, and a control for each repetition). Procedure followed preliminary tests. After counting immovable organisms, temperature (20 ± 2 °C), pH (7.1 – 7.8) and dissolved oxygen (9.2 – 10.6 $mg.L^{-1}$) were registered by U10 water analysis multi-parameter probe (HORIBA, 1991).

At the end of tests, results were subjected to quality and validity controls which consisted of the following items: concentration of dissolved oxygen (DO) at control ≥ 2 $mg.L^{-1}$; number of immovable organisms at control not exceeding 10%; room temperature at 20 ± 2 °C; sensitivity tests with potassium dichromate at the acceptable rate of CE50,24h between 0.9 and 2.0 $mg.L^{-1}$.
Data of acute toxicity tests with regard to mortality rate were analyzed by Trimmed Spearman Karber statistical method (Hamilton et al., 1977) for CE(I)50; confidence intervals (5% and 95%) defined by Rand & Petrocelli (1988) who estimated concentration that caused mortality in 50% of the population exposed to a toxic agent during a certain time period.
CE(I)50 and confidence intervals were given in percentages (DIN 38409, 1989; DIN 38412, 1989; CETESB, 1991; ABNT, 1993) and in acute toxic unit (ATU; 1 TU = 100/CE(I)50), a percentage of sample dilution (Villegas-Navarro et al., 2001; Yu et al., 2003; Emmanuel et al., 2004).

Chronic toxicity tests with *Daphnia magna*

Chronic toxicity tests followed instruction by DIN 38409 (1989), DIN 38412 (1989), CETESB (1991) and ABNT (1993).
Young Daphnia magna were exposed to several sub-lethal concentrations of the effluents during 21 days. Effects of test compound on the organism's survival, growth and reproduction were evaluated during that period.
Preparation of test solutions was similar to that for acute toxicity tests. However, the highest concentration used in chronic toxicity tests was the lowest concentration obtained in the acute toxicity test which caused a contrary effect (immobility) in the organisms.
Ten recipients (250 mL), containing 200mL of test solution, were used for each concentration. One organism per recipient was placed for each concentration, in seven recipients, for survival, growth and reproduction data. The three remaining recipients had five organisms each for survival data. Control with dilution water was prepared for each test. Organisms were carefully, albeit randomly, placed in the test tubes. Feed for Daphnia magna was added to the solutions, and recipients were kept at 20 ± 2 °C. Organisms were counted and measured for total length on the 7th and 21st day of the tests.
Temperature, pH, DO and water electrical conductivity were taken for each test, at the beginning and end of the test, once a week, by U10 water analysis multi-parameter probe (HORIBA, 1991).
Results underwent a quality control for validity. Confidence parameters were: control mortality less than 20%; the production of 40 young organisms in each control group at the end of 21 days; lack of ephippids in control; temperature between 19 and 21°C; DO concentration above 50% saturation.
Data of chronic toxicity tests followed ASTM (1983), known as chronic rate (VC) or the geometrical mean between the highest concentration without a contrary effect (CENO) and the lowest concentration that caused the result observed (CEO) (CETESB, 1991). Moreover, chronic rates were also expressed in chronic toxic unit (CTU) (1 TU=100/VC), or rather, a percentage of the sample's dilution (Emmanuel et al., 2004).

Determination of impact of effluent treated in the receiving body

Evaluation utilized chronic values from assays with Daphnia magna and from the effluent's Concentration in the Receiving Body (CER) posterior to total mixture, following Bassoi et al. (1990) and Caracterização (2000). These authors insisted that criterion CER ≤ UT was recommended so that no chronic effects occurred on the water biota.

$$CER = \frac{\text{Mean discharge of effluent x } 100}{\text{Mean discharge of effluent} + Q_{7,10} \text{ of receiving body}}$$

Q7,10 rates (river's annual minimum discharge, mean rate of 7 days, with probable return in 10 years) were obtained from the site (found on the IBGE map of the municipality, scale 1:10.000) of discharges of liquid effluents in the receiving water bodies, and their dispatch to the CETESB hydrological office of the municipality of São Paulo SP Brazil. Q7,10 rates were thus determined by the hydrological office from data collected from discharge area and from rainfall rates in the region under analysis.

Mean discharges of effluents from the poultry and pig abattoirs were determined and shown in Tables 1 and 2 respectively.

Results were analyzed by Tukey's test at 5% significance level. SAS (1996) was used for statistical analyses, following Steel & Torrie (1960).

Table 1. CE $(I)_{50}$ values, in %, of acute toxic units (ATU) from acute bioassays in the effluents of seven poultry and pig abattoirs, between May and September 2003 (dry period) and January and March 2004 (rainy period) in the state of São Paulo, Brazil.

ABAT.	*Poultry*				*Pig*			
	DRY		*RAINY*		*DRY*		*RAINY*	
	CE(I)$_{50}$	*ATU*	*CE (I)$_{50}$*	*ATU*	*CE(I)$_{50}$*	*ATU*	*CE (I)$_{50}$*	*ATU*
01	45.10	2.21	55.94	1.78	13.07	7.65	30.40	3.28
02	*	1	*	1	25.02	3.99	85.68	1.16
03	*	1	*	1	16.17	6.18	40.22	2.48
04	24.65	4.05	33.23	3.00	9.10	10.98	22.09	4.52
05	65.67	1.52	98.25	1.01	25.99	3.84	70.18	1.42
06	47.32	2.11	74.64	1.33	81.23	1.23	92.68	1.08
07	31.35	3.18	42. 37	2.36	88.21	1.13	98.45	1.02

Table 2. Concentration of effluents in the receiving body (CER), chronic values (VC) and chronic toxic units (CTU) from chronic bioassays in the effluents of seven poultry and pig abattoirs between May and September 2003 (dry period) and January and March 2004 (rainy period) in the state of São Paulo, Brazil.

ABAT.	*Poultry*					*Pig*				
	CER	*DRY*		*RAINY*		*CER*	*DRY*		*RAINY*	
		VC	*CTU*	*VC*	*CTU*		*VC*	*CTU*	*VC*	*CTU*
01	0.365	31.62	3.16	17.81	5.61	RP	2.39	41.84	4.18	23.92
02	0.510	79.06	1.26	47.24	2.11	0.346	63.46	1.57	73.28	1.36
03	0.273	79.06	1.26	72.17	1.38	0.043	10.54	9.48	17.40	5.74
04	26.405	14.80	6.75	13.98	7.15	0.123	2.31	43.29	3.17	31.54
05	0.027	23.57	4.24	22.36	4.47	55.068	2.00	50.00	13.36	7.48
06	6.666	26.54	3.76	15.42	6.48	22.071	56.15	1.78	67.15	1.48
07	17.023	16.84	5.93	15.62	6.40	1.267	22.36	4.47	44.90	2.22

Table 3. Characterization of poultry abattoirs (ABT) from which samples were collected between May and September 2003 (dry period) and between January and March 2004 (rainy period) in the interior of the state of São Paulo, Brazil.

	ABT 1	*ABT 2*	*ABT 3*	*ABT 4*	*ABT 5*	*ABT 6*	*ABT 7*
Inspection	State	State	State	Federal	Federal	State	Federal
animals slaughtered/dia	24,000	13,000	5,000	135,000	16,128	8,000	105,000
Blood recuperation	Yes	yes	yes	yes	yes	yes	Yes
workers (n)	130	80	25	738	70	80	480
Disposal of human excrement	Stream	cesspit	cesspit	Municipal treatment station (MTS)	Abattoir treatment station	MTS	Cesspit
Source of water used in abattoir	well depth > 20m	well depth > 20 m	well depth > 20 m	well depth > 20 m	well depth > 20 m	Well depth > 20 m	Source and well depth > 20 m
Treatment of affluent used	Initial phase	Primary treatment; secondary treatment (2 aerobic stabilizing pools and 1 refining lake; stream.	Primary treatment; secondary treatment (3 stabilizing lakes); irrigation.	Primary treatment; secondary treatment (2 aerobic stabilizing lakes and 1 anaerobic lake); stream.	Primary treatment; secondary treatment (1 alternative stabilizing lake and 1 anaerobic lake); stream.	Primary treatment; secondary treatment (1 aerobic stabilizing lake); stream.	Primary treatment; secondary treatment (3 anaerobic stabilizing lakes); stream.
Effluent's volume (yearly mean)	720 m^3/day	200 m^3/day	75 m^3/day	2.400 m^3/day	458 m^3/day	120 m^3/day	1.575 m^3/day
Class of receiving body	4	2	No information	3	2	2	2
Cleanliness (pre-washing, detergent and hygienization)	Daily	Daily	Daily	Daily	Daily	Daily	Daily

Table 4. Characterization of pig abattoirs (ABT) from which samples were collected between May and September 2003 (dry period) and between January and March 2004 (rainy period) in the interior of the state of São Paulo, Brazil.

	ABT 1	*ABT 2*	*ABT 3*	*ABT 4*	*ABT 5*	*ABT 6*	*ABT 7*
Inspection	State	Federal	State	Federal	State	Federal	Federal
Slaughtered animals/day	60	250	35	110	150	700	1.000
Blood recuperation	yes	yes	no	Yes	yes	yes	Yes
workers (n)	12	68	10	80	13	92	286
Disposal of human excrement	Municipal treatment station	cesspit	cesspit	Municipal treatment station	cesspit	cesspit	Cesspit
Source of water used in abattoir	Well depth > 20 m	Well depth > 20 m	Well depth > 20 m	Well depth > 20 m	Well depth > 20 m	Well depth > 20 m	Well depth > 20 m
Treatment of affluent used	Municipal treatment station	Primary treatment; secondary treatment (5 alternative stabilizing lakes); irrigation.	Primary treatment; secondary treatment (1 alternative stabilizing lake); irrigation.	Primary treatment; secondary treatment (1alternative stabilizing lake); stream.	Primary treatment; secondary treatment (1 alternative stabilizing lake); irrigation.	Primary treatment; secondary treatment (1 anaerobic stabilizing lake and 3 alternative lakes); stream.	Primary treatment; secondary treatment (2 anaerobic stabilizing lakes, 1 aerobic lake, 1 drying lake and 1 level lake); stream.
Effluent's volume (yearly mean)	12 m^3/day	100 m^3/day	17 m^3/day	55 m^3/day	90 m^3/day	350 m^3/day	500 m^3/day
Class of receiving body	2	No information	No information	2	2	2	2
Cleanliness (pre-washing, detergent and hygienization)	weekly	daily	daily	daily	weekly	daily	Daily

RESULTS AND DISCUSSION

Table 1 shows CE (I)50 and ATC rates calculated for acute toxicity tests from effluents of treatment systems of poultry and pig abattoirs during the dry and rainy seasons.

It is important to remind that similar results have already been obtained by Gherardi-Goldstein et al. (1985), Abbasi et al. (1988), Bertoletti et al. (1989), Bervoets et al. (1996), Caracterização (2000) and Hongxia et al. (2004).

According to Bassoi et al. (1990) and Caracterização (2000), CE(I)50 values characterize an inverse relationship of the water sample's toxicity, or rather, toxicity will be as low as dilution. The above authors also state that the relation of TU is proportional, or rather, a higher TU rate means higher toxicity. Villegas-Navarro et al. (2001) also declared that ATU $\leq$ 1 is non toxic; > 1 is toxic; $\geq$ 3 highly toxic.

According to above authors, treatment systems of effluents from the fourth poultry abattoir (Table 1) during the dry period were the most toxic in terms of CE (I)50 (24.65 %) and ATU (4.05) rates. On the other hand, abattoir 5 had the less toxic effluent (CE(I) 50 = 65.67% and ATU = 1.52). The same trend was maintained during the rainy period, with a slight decrease in toxicity. In fact, CE(I) 50 and ATU rates of abattoir 4 increased to 33.23% and decreased to 3.00, respectively; in abattoir 5 rates were 98.25% and 1.01 respectively.

Highest toxicity in pig abattoirs (Table 1) was reported in the treatment systems of abattoir 4 with CE (I) 50 = 9.10% and ATU = 10.98 during the dry period and CE (I) 50 = 22.09% and ATU = 4.52 during the rainy period. A slight decrease in toxicity level was detected during the period. On the other hand, less toxic affluents originated from the treatment system of abattoir 7. During the dry period (CE (I) 50 = 88.21 % and ATU = 1.13), toxicity was higher than that reported during the rainy season (CE (I) 50 = 98.45 % and ATU = 1.02).

According to CETESB (1987) and Caracterização (2000), a toxicity scale, in use in Brazil, classifies samples in most toxic (CE50 $\leq$ 25 %), toxic (CE50 25 – 50 %), moderately toxic (CE50 51 – 75 %), slightly toxic (CE50 76 – 99 %) and non toxic (CE50 $\geq$ 100 %). During the dry period, one (14.28%) poultry abattoir was classified as highly toxic (Abattoir 4); three (42.85%) as toxic (Abattoirs 1, 6 and 7) and one (14.28%) as moderately toxic (Abattoir 5) and two (28.57%) as non toxic (Abattoir 2 and 3). During the rainy period slight decreases in toxicity levels were detected. Two effluents (28.57%) were considered toxic (Abattoir 4 and 7); two (28.57%) moderately toxic (Abattoir 1 and 6), one (14.28%) slightly toxic (Abattoir 5) and two (28.57%) non toxic (Abattoir 2 and 3).

During the dry period, effluents from four pig abattoirs (57.14%) were highly toxic (Abattoir 1, 2, 3 and 4), one (14.28%) toxic (Abattoir 5) and two (28.57%) slightly toxic (Abattoir 6 and 7). However, during the rainy period, one effluent (14.28%) was highly toxic (Abattoir 4), two (28.57%) toxic (Abattoir 1 and 3), one (14.28%) moderately toxic (Abattoir 5) and three (42.85%) slightly toxic (Abattoir 2, 6 and 7).

Whereas two poultry abattoirs presented non toxic effluents during the dry and rainy periods, Villegas-Navarro et al. (1997) studied the toxicity of effluents in food industries and found residual waters with ATU less than 1, which corroborated our findings

Table 2 shows effluent concentrations of the receiving body (CER), chronic rates (VC) and chronic toxic units (CTU) from chronic bioassays, coupled to effluents of treatment systems from poultry and pig abattoirs during the dry and rainy periods.

Following Bassoi et al. (1990) and Caracterização (2000), the criterion CER ≤ CTU is recommended to avoid chronic effect on aquatic biota. Three effluents samples (Abattoirs 4, 6 and 7) from poultry abattoirs which were treated and discharged in water bodies Class II and III (SÃO PAULO, 1976; CONAMA, 2005) during the dry and rainy periods had values consonant to CER ≥ UTC. Consequently, they were the only ones which could support the chronic impact to water organisms of the receiving water bodies. In the case of pig abattoir treatment systems, only two effluents (Abattoir 5 and 6), in the dry and rainy season, presented chronic impact on aquatic life, complying with CER ≥ UTC.

According to Muna et al. (1995) and Yang et al. (1999), when chemical analysis give limited information on the pollution of residual discharges, biological tests become efficient as far as they reveal residual water's toxicity. However, the same authors insist that information on the effluents' chemical composition is an important item in ecotoxicological procedures.

In spite of the high ATU and CTU rates in the current research, it may be stated that efficiency of some residual treatment stations should be improved. The above issue raises the point on whether the responsibility with regard to environmental impact lies on the industry that discharges liquid residues or on the company that built the treatment station and warranted the absence of toxic residual water.

Toxicity tests either with Daphnia magna or with other organisms are an indispensable tool to foresee the impact that industrial affluents cause to the biota of water bodies and the allowable toxicity level to avoid such an occurrence.

Conscientious economical growth as a long term policy, underscoring the principle of shared responsibility in environmental programs, may put an end to the conflict between industries and environmental institutions. A time will come in which dialogues will cause responsibility in environmental protection and contribute towards sustainable development.

CONCLUSION

Within the context of the fourteen abattoirs under analysis, chronic toxicity tests with *Daphnia magna* showed that effluents of three poultry and two pig abattoirs might cause an environmental impact during the dry and rainy season.

AKNOWLEDGEMENTS

The authors would like to thank the Research Foundation of the State of São Paulo (FAPESP) for the scholarship and the financing of the project.

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