Catalase activity in *Smicridea* McLachlan, 1871 (Insecta, Trichoptera) collected from natural and altered/impacted streams

Atividade da catalase em *Smicridea* McLachlan, 1871 (Insecta, Trichoptera) coletados em riachos naturais e alterados/impactados

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Abstract: Aim: We compare catalase activity in *Smicridea* McLachlan, 1871 (Insecta, Trichoptera) collected in natural and agricultural streams and correlates the enzyme pattern with metal content in the water. **Methods:** Organisms were collected in sites classified as natural (riparian vegetation in buffer zone) and altered/impacted (agricultural land use in drainage area) environments, located at Cravo River and Campo River sub-basins (RS, Brazil). Next the collected larvae were identified and used to proteins quantification and catalase activity measure. The concentration of Mg, Cr, Cu, Pb and Cd in the water was determined by atomic absorption spectrometry. **Results:** Catalase activity in *Smicridea* ranged from 1.5 to 6 U, with mean values about 2.63 ± 0.096 U (SEM). The presence of metals was higher in the streams located at agricultural drainage area, except for Mg at the Cravo sub-basin and Cu at the Campo sub-basin. Catalase was higher in *Smicridea* collected in natural streams as compared to that agriculture streams and was correlated with Pb and Cd levels. **Conclusions:** The data showed the potential of this biomarker as a useful tool for complementation of water quality biomonitoring studies using *Smicridea* as bioindicator.

Keywords: biomarkers; biomonitoring; *Smicridea*; cadmium; lead.

Resumo: Objetivo: Foi comparada a atividade da catalase em *Smicridea* McLachlan, 1871 (Insecta, Trichoptera) coletados em riachos margeados por vegetação ripária ou por atividades agrícolas e correlacionado o padrão da enzima com o conteúdo de metais na água. **Métodos:** Os organismos foram coletados em pontos classificados como naturais (margeados por vegetação ripária) ou alterado/impactados (margeados por atividades agrícolas), localizados nas sub-bacias do Rio do Campo e do Rio do Cravo (RS, Brasil). Em seguida foram identificados e usados para determinação de proteínas e medida da atividade da catalase. A concentração de Mg, Cr, Cu, Pb e Cd na água foi determinada por espectrometria de absorção atômica. **Resultados:** A atividade da catalase em *Smicridea* variou entre 1,5 e 6 U, com valores médios de 2,63 ± 0,096 U (EP). A presença de metais foi maior nos riachos localizados na matriz agrícola, exceto para o Mg na sub-bacia do Rio Cravo e Cu na sub-bacia do Rio Campo. A atividade da catalase foi maior em *Smicridea* coletados nos locais margeados por vegetação ripária em relação aos margeados por agricultura, e foi negativamente correlacionada com os níveis de Pb e Cd. **Conclusões:** Os dados mostram o potencial da catalase como ferramenta complementar em estudos de biomonitoramento da qualidade de água usando *Smicridea* como bioindicador.

Palavras-chave: biomarcadores; bioindicadores; *Smicridea*; cádmio; chumbo.

Biomarkers are defined as alterations at cellular, physiological or biochemical level in response to a stress condition. In general, biomarkers responses tend to precede changes in the composition and structure of the communities (Holt & Miller, 2010). Specifically, biomarkers of oxidative stress detect alterations resulting from increased exposure to oxidant agents or reduction in antioxidant defenses.

Catalase, a well-established biomarker, is an essential enzyme of antioxidant defense system, which is present virtually in all aerobic organisms. This enzyme catalyzes the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen. A wide variety of stressors encountered in aquatic environments is able to alter the levels of catalase activity (Chandran et al., 2005; Mena et al., 2014).

Oxidative biomarkers are widely applied in biomonitoring realized with fish species (Mena et al., 2014). In benthic macroinvertebrates the analysis of oxidative stress parameters is less widespread, but there are works with promising results in this field (Berra et al., 2004; Prat et al., 2013). The objective of this work was compare catalase activity of *Smicridea* McLachlan, 1871 (Insecta, Trichoptera) collected in natural and impacted streams. *Smicridea* is a water quality bioindicator with generalist habit which can be found in environments with different degrees of preservation (Bentes et al., 2008). Our hypothesis is that catalase activity can distinguish *Smicridea* from natural environments those collected from impacted or altered environments. In addition, we also hypothesized that enzyme pattern correlates with heavy metal content in the water.

Collects were made in Campo River and Cravo River hydrographic sub-basins, belonging to Alto Uruguai River of Rio Grande do Sul (Figure 1). In each sub-basin were delimited sampling sites bordered by riparian vegetation (natural streams) and by agricultural land use in drainage area (impacted streams), which presented distinct classification based on rapid assessment of the habitats diversity protocol (Callisto et al., 2002). At least three independent collects per site were performed between March and June of 2012. Organisms were captured with a Surber sampling (mesh: 250 μm; area: 0.09 m^2), transported alive to the Biomonitoring Laboratory (URI – Erechim – RS) and identified (Pes et al., 2005). Biological extracts were obtained by maceration of 6-10 organisms as described by Bertholdo-Vargas et al. (2009) and was used to protein quantification (Bradford, 1976) and catalase activity measure (Bertholdo-Vargas et al., 2009). The biochemical analyzes were made at least in triplicate. The assays of metals in the water were made by atomic absorption spectrometry, following the protocol described in APHA (1998). To evaluate differences in catalase activity between organisms collected in agricultural and natural streams a student t test was performed. The comparison between concentrations of metals in all sampling sites was made by ANOVA plus Tukey post test. To investigate the correlation between metal content and catalase activity was used a Linear Pearson correlation.

In benthic macroinvertebrates the levels of catalase activity differ broadly from taxa to taxa, range from 3 to 100 U (Berra et al., 2004), however, specific data about these enzyme in *Smicridea* are not available in the literature. In these work, the level of catalase in *Smicridea* ranged from 1.5 to 6 U, with general mean values about 2.63 ± 0.09 U (SEM).

The results about metals showed that Cu, Pb and Cd were present in high concentrations, overcoming

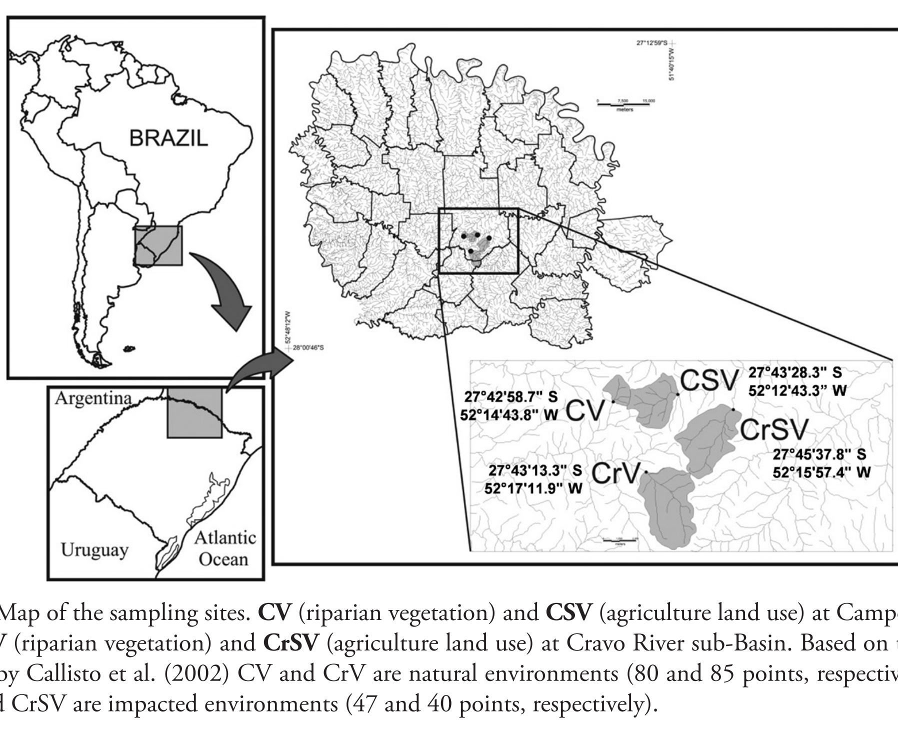

Figure 1. Map of the sampling sites. **CV** (riparian vegetation) and **CSV** (agriculture land use) at Campo River sub-Basin. **CrV** (riparian vegetation) and **CrSV** (agriculture land use) at Cravo River sub-Basin. Based on the protocol described by Callisto et al. (2002) CV and CrV are natural environments (80 and 85 points, respectively); CS are altered and CrSV are impacted environments (47 and 40 points, respectively).

the upper limit established by (Brasil, 2005), as exception of Cd in the natural streams (Table 1). In general, the level of metals was higher in the agricultural streams, except for Mg in the Cravo sub-basin and Cu in the Campo sub-basin.

In agreement, catalase activity was higher in *Smicridea* collected in streams bordered by riparian vegetation as compared to that bordered by agriculture (Figure 2). These data can be related to the differences in Pb and Cd at distinct sampling sites (Table 1), since a strong negative correlation between these metals and catalase was observed (r = -0.99, $p = 0.008$ to Pb and r = -0.95, $p = 0.041$ to Cd). For Mg and Cu was not correlation.

Catalase inhibition by Cd was described in invertebrates as snail *Achatina fulica* and crab *Sinopotamon henanense* (Chandran et al., 2005; Wu et al. 2013). This inhibition can be result of Cd-linkage in specific sites of catalase structure, which is critical in relation to their function (Casalino et al., 2002). In the same way, it was described that high concentrations of Pb can decrease catalase in the snail *Theba pisana* treated directly with the metal as well in *Lymnaea natalensis* exposed to sediment and water contaminated by Pb (Radwan et al., 2010; Siwela et al., 2010).

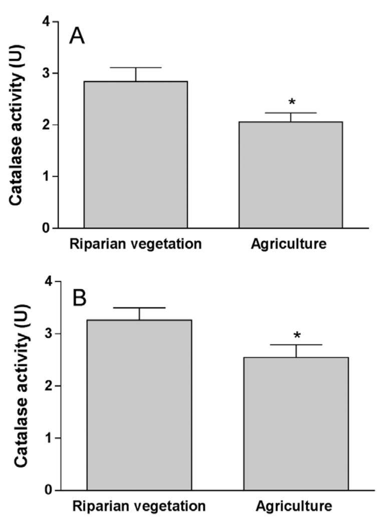

Figure 2. Catalase activity in *Smicridea*. **(A)** Cravo River sub-basin, **(B)** Campo River sub-basin. The data are presented as mean (± SEM). One unit of catalase activity (U) corresponds to the enzyme necessary to degrade H_2O_2 (µMol min^{-1} mg^{-1} protein). *Indicate $p < 0.05$, as analyzed by student t test.

Some studies point to the importance of biomarkers inclusion in protocols that analyze environmental pressures on macroinvertebrates communities in polluted rivers (Prat et al., 2013). In these sense, catalase, as well another biomarkers of oxidative stress, is responsive to several environmental pollutants as metals, hydrocarbons and pesticides (Van Der Oost et al., 2003; Barata et al., 2005), so it can be used as a general indicator of water quality in contaminated streams by complex mixtures of xenobiotics. In cellular and physiological terms, the inhibition of catalase increase the H_2O_2 availability, which by reaction with metals as iron and copper, can generate reactive molecules as hydroxyl radical ($OH^•$). The latter is highly harmful to the living organisms, since it is able to damage essential biomolecules as DNA, proteins and lipids (Halliwell & Gutteridge, 2007). Therefore, detect changes in catalase and other oxidative biomarkers, is a manner of identify prematurely environmental/ecological perturbations that often are not yet expressed at populations or communities level.

This work presents a first description of catalase activity in *Smicridea*, which was correlated with Pb and Cd content in sampling sites with different conservation status. The work confirms the efficiency of catalase as complementary tool for water quality biomonitoring based on macroinvertebrates bioindicators. Additional efforts are necessary to the standardization of other biomarkers measure in *Smicridea*, as well in other bioindicators of water quality natives from Brazil.

Table 1. Concentrations of some metals in the water of Cravo and Campo sub-basins.

	Cravo river sub-basin		Campo river sub-basin		Upper limit*
	Riparian vegetation	Agriculture	Riparian vegetation	Agriculture	
Mg (µg.L^{-1})	125.0^b ± 7.0	150.0^b ± 8.0	132.0^b ± 1.0	348.0^a ± 12.0	ND**
Cr (µg.L^{-1})	0.0	0.0	0.0	0.0	50.0
Cu (µg.L^{-1})	21.0^c ± 3.0	210.0^a ± 2.0	130.0^b ± 0.2	23.0^c ± 0.2	9.0
Pb (µg.L^{-1})	240.0^b ± 9.0	273.0^a ± 17.0	216.0^b ± 14.0	259.0^a ± 12.0	10.0
Cd (µg.L^{-1})	1.0^c ± 0.2	6.0^a ± 0.2	0.0^d ± 0.0	4.0^b ± 0.3	1.0

Different letters indicate statistical differences ($p < 0.05$) comparing the same metal at the four sampling sites, as analyzed by ANOVA plus Tukey test. *Upper limit in relation to reference values established by CONAMA resolution (Brasil, 2005). **Upper limit not determined by CONAMA (Brasil, 2005).

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