# SRAS Final Report: *Caiman latirostris* as an indicator of heavy metals present in environments with different levels of anthropization.

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## Abstract

Agricultural activity has increased in recent years in our country due to the global demand for resources, causing multiple and contradictory impacts from an environmental perspective. The use of agrochemicals is a common practice in rural areas, which leads to the dispersion of heavy metals (HM) associated with their formulation. The caiman (*Caiman latirostris*) is an animal widely distributed within Santa Fe province, and has a high probability of exposure to pollutants at all stages of its life because it is at the top of the food chain, long-lived and faithful to its habitat. These characteristics support its selection as a sentinel for alerting the levels of pollutants in five Santa Fe wetlands. In order to evaluate the impact of anthropogenic activities on wetlands in Santa Fe, we determined the concentrations of HM present in *C. latirostris* tissues in sites with different degrees of anthropization. From our results, we confirmed the presence of heavy metals in all five sites with differences between sites in terms of the metal found and its concentrations but the highest concentrations were not related to the most anthropized place. In addition, there were differences between the tissues, with higher concentrations in muscle than in blood.

## Introduction

Agricultural activity has been increasing in recent years in Argentina due to the global demand for resources, particularly food, as a consequence of demographic expansion. This advance of the agricultural frontier over natural environments has increased due to the use of new technologies. Innovations in agricultural activity such as the improvement or genetic modification of crops, or the appearance of no-till farming and new families of agrochemicals and fertilizers, have had multiple and often contradictory impacts from an environmental perspective (Jobbágy, 2010; Di Marzio et al., 2019). Another important vector of agricultural transformations is the changed environment, and especially in the case of central Argentina in the last three decades the increase in pluviometric flows (Jobbágy, 2010). This leads to the dispersion of various pollutants, such as heavy metals associated with the use of pesticides and fertilizers (Carnelo et al. 1997; Salazar et al., 2012), related to agricultural practices (Jobbágy, 2010; Di Marzio et al., 2019; Adimalla et al., 2019; Tavalieri et al., 2020; Napoletano et al., 2023).

Heavy metals (HM) can be defined as any metallic chemical element that has a high density, is toxic or poisonous at even very low concentrations (Lucho et al., 2005) and has adverse effects on the health of organisms (Buenfil-Rojas and Flores-Cuevas, 2007; Stark et al., 2022). Heavy metals or metalloids include cadmium (Cd), arsenic (As), lead (Pb), copper (Cu) and zinc (Zn) among others (Lucho et al., 2005). In the last 50 years, heavy metal pollution has increased, considerably affecting various ecosystems (Schneider et al., 2013; Turkyilmaz et al., 2018; Oliveira et al., 2021). These compounds are not biodegradable, so they persist, accumulating in the environment and biomagnify within organisms as they pass through the different trophic levels that make up an ecosystem via the food chain (García-Fernández et al., 2005; Schneider et al., 2013). The effects caused by these metals on fauna depend on the concentration to which the organisms are exposed. The biological processes most affected by their presence include: cellular respiration, lipid and carbohydrate metabolism, hormone synthesis and secretion, reproduction and growth (Fingerman et al., 1996; Buenfil-Rojas, 2014; Gholamhosseini et al., 2021). Heavy metals in reptiles have been widely recognised as one of the greatest threats to these populations (Smith et al., 2007). The exposure pathways to which these individuals are subjected are varied, and they can absorb heavy metals through accidental or voluntary

ingestion, dermal contact, inhalation, maternal and even through the environment in which they live (Smith et al., 2007; Schneider et al., 2013; Lemaire et al., 2021; Humphries et al., 2022).

Wild animals can be considered as a bioindicator of environmental risks present in the ecosystem (Fox, 2001; Parra, 2014). In reptiles, there is evidence that high levels of heavy metals may be affecting their metabolism, growth rate and reproductive success (Delany et al., 1988; Fingerman et al., 1996; Piña et al., 2009), a relationship that has also been observed in birds (Nam and Lee, 2006; Malik and Zeb, 2009). Exposure pathways are varied being able to absorb HM by ingestion, dermal contact, inhalation, maternal transfer and even through the environment in which the nests are located. In the last 30 years, several studies have reported the presence of heavy metals in different crocodilian species (Delany et al., 1988; Campbell et al., 2010; Schneider et al., 2013). In addition, the extraordinary capacity of these reptiles to accumulate these metals has been studied, as well as possible disposal routes to avoid poisoning (Xu et al., 2006). The caiman (*Caiman latirostris*) is widely distributed in the region, and has a high probability of exposure to contaminants at all stages of its life because it is at the top of the food chain, long-lived and habitat-loving (Larriera and Imhof, 2006). All these physiological and ecological characteristics are conducive to their selection as sentinels, that is caimans will be able to warn about the presence of pollutants (Poletta, 2011; Böhm et al., 2013). The concentration of contaminants in the tissues of these sentinel organisms would be evidence of the presence of these metals in the habitat (Lailson-Brito et al., 2002). Thus, our goals were to determine the concentrations of HM present in blood and muscle of *Caiman latirostris* in study sites with different degrees of anthropization.

## Methods

During the period 2019-2022, we took samples from *Caiman latirostris* individuals of different sizes and sexes. Samples were collected from sites with different degrees of anthropization as defined from the wide range vegetation index of Gitelson (2004), according to Viña et al. (2008), obtained from the Centro Regional de Geomática (CEREGEO). The different sites corresponded to Proyecto Yacaré survey areas in the north of Santa Fe province: Reserva “El Estero” (San Javier), Estancia “El Cacique” (San Justo), El Espín (Vera), Reserva Natural Manejada “El Fisco” and Costa del Salado (San Cristóbal). Being the most anthropized site El Espín and the least anthropized Costa del Salado (Representation of the most anthropized at least: El Espín > El Estero> El Cacique / El Fisco > Costa del Salado).

In order to achieve the proposed objective, 50 samples were taken from both females and males, juvenile and adult. Wild animals were captured using steel ropes. After capture, individuals were physically immobilized without the use of anesthetics (Verdade, 1997; Figure 1). Captured individuals were sexed by inspection of the cloaca, as described Brazaitis (1969) and Yanosky (1990). Biometric data were obtained with a tape (1 mm accuracy) by measuring the total length (TL), snout-vent length (SVL) and weighed with a scale of 0.01 kg precision. Subsequently, the individual was released.

For the proposed objective, biological samples (blood and muscle) were taken. Blood samples (approximately 6 mL) were taken from the dorsal postcranial sinus according to the technique of Portelinha et al. (2015), and stored in vacutainer tubes with sodium heparin. Muscle samples (approximately 2 $cm^2$ skin sample by cutting the single scale of the tail, according to the markings on the tails of the caimans) are collected in zip lock bags. Both methods are commonly used to mark crocodilians, and are not considered harmful (Marques et al., 2013). In all cases, samples were kept refrigerated at 4ºC until further processing in the laboratory.



**Figure 1:** Immobilization of caiman, previously to take the blood and muscle samples.

## Results

The non-parametric Kruskal Wallis test was performed for muscle and blood HM values because the datasets did not meet the assumptions of normality and homoscedasticity.

Within the muscle, there was no statistical difference found between the study sites for Cu ($p = 0.15$) or Zn ($p = 0.21$; Table 1). For Ni and As, sites El espín and El fisco were significantly lower than sites El cacique, El estero, and Costa del Salado ($p < 0.01$). El fisco had significantly higher Cr levels than El estero and Costa del Salado which were significantly higher than El cacique and El espín. El fisco did not have any detectable As whilst the other sites all had comparable levels. Similarly, site El fisco had the lowest ($p < 0.001$) Pb level compared to the other sites. Sites El estero and El espín had significantly lower muscle Cd levels than the other sites ($p < 0.05$; Table 1).

**Table 1.** Concentrations of metallic elements (mean ± standard deviation) detected in *Caiman latirostris* muscle from the different study sites with different degrees of anthropization. For statistical analyses, the "non-detectable" values will be taken as zero values to apply the corresponding statistic. Different superscripts indicate a significant difference at the 5% level ($p<0.05$).

| Site | Cu (µg/Kg) | Ni (µg/Kg) | Cr (µg/Kg) | As (µg/Kg) | Pb (µg/Kg) | Zn (µg/Kg) | Cd (µg/Kg) |
|---|---|---|---|---|---|---|---|
| El cacique | 395.5 ± 260.1 | 233.3 ± 234.1 $^{B}$ | 59.6 ± 75.7 $^{AB}$ | 68.3 ± 73.4 $^{B}$ | 59.5 ± 52.8 $^{B}$ | 45416.7 ± 21360.4 | 11.2 ± 15.7 $^{B}$ |
| El estero | 330.9 ± 126.2 | 193.4 ± 182.0 $^{B}$ | 101.8 ± 123.2 $^{AB}$ | 29.3 ± 23.5 $^{B}$ | 36.2 ± 30.4 $^{B}$ | 41000.0 ± 10253.9 | 2.1 ± 3.2 $^{A}$ |
| Costa del Salado | 507.6 ± 251.4 | 337.1 ± 275.9 $^{B}$ | 100.5 ± 67.2 $^{B}$ | 114.9 ± 130.9 $^{B}$ | 47.4 ± 37.5 $^{B}$ | 51636.4 ± 25527.5 | 2.5 ± 2.0 $^{B}$ |
| El espín | 320.0 ± 107.6 | 110.5 ± 59.1 $^{AB}$ | 45.9 ± 57.8 $^{A}$ | 54.6 ± 43.3 $^{B}$ | 13.7 ± 6.8 $^{B}$ | 34545.5 ± 16687.5 | 0.5 ± 0.4 $^{A}$ |
| El Fisco | 465.0 ± 201.8 | 36.4 ± 72.9 $^{A}$ | 282.8 ± 132.7 $^{C}$ | 0.0 $^{A}$ | 6.7 ± 15.2 $^{A}$ | 31875.0 ± 12631.5 | 29.2 ± 31.4 $^{B}$ |
| *p*-value | 0.15 | 0.01 | 0.001 | 0.001 | 0.0001 | 0.21 | 0.05 |

In blood, there was no statistical difference found between the study sites for Zn ($p$ = 0.33; Table 2). At El fisco, As and Pb were not detected, while at the other sites the levels were comparable with the exception of site Costa del Salado, which presented the highest values. For Ni and Cr it was not possible to apply a statistical analysis because the sampling was not representative, since it was found in one or two animals.

**Table 2.** Concentrations of metallic elements (mean ± standard deviation) detected in blood of *Caiman latirostris* of different sexes and sizes, in study sites with different degrees of anthropization. For statistical analyses, the "non-detectable" values will be taken as zero values to apply the corresponding statistic. Different superscripts indicate a significant difference at the 5% level ($p$<0.05).

| Site | Cu (µg/L) | Ni (µg/L) | Cr (µg/L) | As (µg/L) | Pb (µg/L) | Zn (µg/L) |
|---|---|---|---|---|---|---|
| El cacique | 267.4 ± 70.4$^{A}$ | 7.1 ± 16.8 | 0.0 | 20.0 ± 18.7 $^{B}$ | 38.7 ± 48.9 $^{B}$ | 2219.1 ± 450.1 |
| El estero | 295.1 ± 105.2$^{A}$ | 14.9 ± 42.0 | 0.5 ± 1.4 | 9.6 ± 10.5 $^{B}$ | 67.0 ± 92.0 $^{BC}$ | 1813.4 ± 690.7 |
| Costa del Salado | 457.8 ± 105.9$^{B}$ | 21.0 ± 48.9 | 0.0 | 32.0 ± 29.3 $^{B}$ | 154.2 ± 308.6 $^{C}$ | 2092.6 ± 333.8 |
| El espín | 330.4 ± 63.8$^{A}$ | 2.8 ± 9.4 $^{A}$ | 1.2 ± 3.9 | 22.8 ± 8.1 $^{B}$ | 30.0 ± 22.4 $^{B}$ | 1862.3 ± 429.1 |
| El fisco | 319.0 ± 115.0$^{A}$ | 0.0 | 0.0 | 0.0 $^{A}$ | 0.0$^{A}$ | 2189.1 ± 481.5 |
| $p$-value | 0.002 | - | - | 0.0004 | 0.0001 | 0.33 |

## Discussion

Successive predation through the food web allows organic and inorganic elements to accumulate and increase concentration in the tissues of organisms, especially at higher trophic levels (Téllez and Merchant, 2015). Therefore, higher level predators generally possess higher concentrations of heavy metals compared to lower trophic organisms (Téllez and Merchant, 2015).

In this study, we analyzed the presence of heavy metals in different tissues (blood and muscle) of *Caiman latirostris* at different sites with different levels of anthropization. Our data show a large variation in metal concentrations among individual caimans. We detected a greater accumulation of HM in muscle than in blood. This is related to the fact that the accumulation in tissue indicates a

prolonged exposure to these metals and in blood the metal(s) circulate which would indicate a recent exposure to these element(s).

We detected Pb concentrations in few caiman but these were elevated, which would indicate that these animals could have ingested organisms contaminated by lead bullets. Camus et al. (1998) found that lead poisoning due to accidental ingestion of lead bullet fragments from otters (*Myocastor coypus*) was a major cause of disease in captive-bred juvenile alligators. Seltzer et al. (2006) also found elevated Pb levels in alligators and attributed this to accidental exposure to Pb from their diets. Wild caimans with high Pb concentrations could be feeding in an area where humans routinely hunt or fish and may have recently fed on an animal containing a lead bullet or fishing sinkers. Numerous waterfowl and other birds are known to ingest lead and succumb to lead poisoning (Scheuhammer and Norris, 1996) could serve as food for caimans.

We found that the arsenic value found in some caimans (e.g., those in group 3) was high relative to other caimans, and this may reflect local environmental factors. Cadmium was detected only in tissue but reflects its presence in all the sites under study. Arsenic exposure has been shown to cause decreased growth (Eisler, 1994), and Cd has been shown to cause reproductive problems (Lemly, 1993). Although some caimans had higher concentrations of As and Cd than other caimans in this study, very high concentrations were not found.

In conclusion, we have detected the presence of different heavy metals in both tissue and blood of *Caiman latirostris* in different study sites but no relationship in HM level was found with the degree of anthropization.

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