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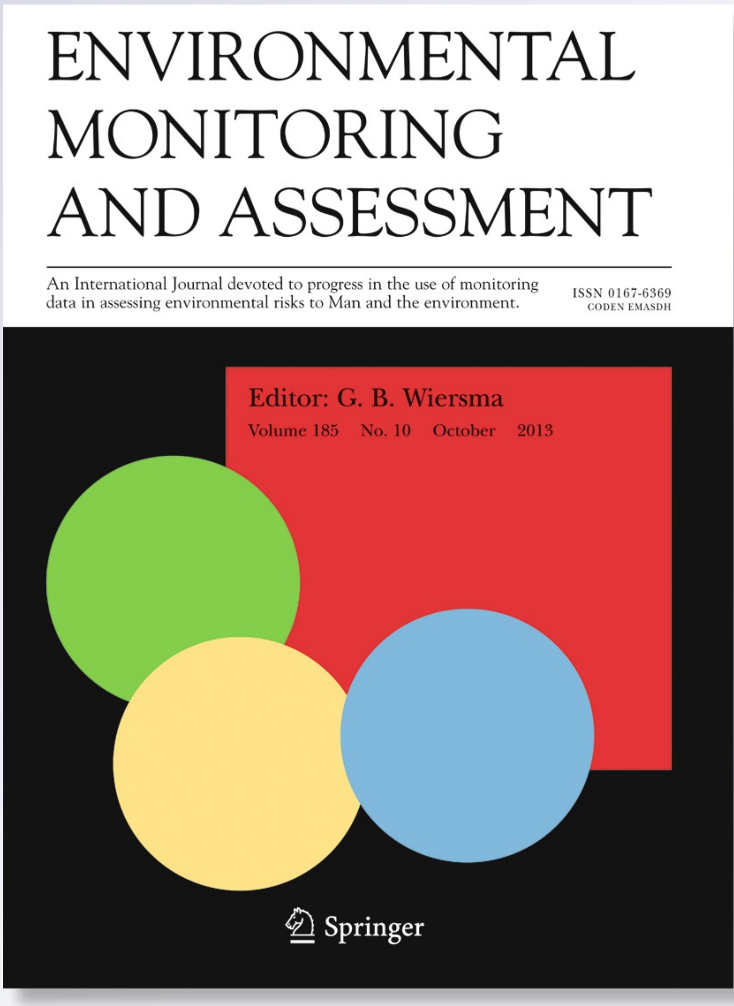

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Habitat monitoring and genotoxicity in *Ucides cordatus* (Crustacea: Ucididae), as tools to manage a mangrove reserve in southeastern Brazil

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Abstract In Brazil, the state of São Paulo contains both preserved areas (Juréia-Itatins Ecological Station) and extremely impacted ones (Cubatão Municipality). This study evaluated the concentrations of five metals (Cu, Cd, Cr, Pb, and Hg) in two mangroves with different levels of anthropogenic impact and the apparent genotoxicity to *Ucides cordatus*. Water and sediment samples were obtained, and metal concentrations were determined with an atomic absorption spectrophotometer. The genotoxic impact was quantified based on the number of micronucleated cells per 1,000 analyzed (MN‰), using hemolymph slides stained with Giemsa. Metal concentrations in water were below the detection limit, except for lead, although no significant difference was observed between the areas ($P>0.05$). Sediment from Cubatão had higher concentrations of Cd, Pb, Cr, and Cu than sediment from Juréia-Itatins ($P<0.05$), but no significant differences in metal concentrations were detected among depth strata of the sediment ($P>0.05$). Crabs from Cubatão had a 2.6 times higher mean frequency of micronucleated cells (5.2 ± 1.8 MN‰) than those from Juréia-Itatins (2.0 ± 1.0 MN‰; $P<0.0001$). The more-polluted conditions found in the mangrove sediments of Cubatão were reflected in the micronucleus assay, demonstrating their genotoxic effect; however, genetic damage should be attributed to a synergistic effect with other kinds of pollutants previously recorded in different environments of Cubatão. *U. cordatus* proved to be an excellent bioindicator of mangrove pollution. This study established, for the first time, the normal frequency of MN‰ in a population of this species within an ecological station.

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Introduction

The rapid growth of the human population and the many and varied human impacts have affected natural environments worldwide (Oliveira et al. 2008). This history reinforces the importance of natural conservation units in providing optimum survival conditions for biota that are more sensitive to human actions (Kramer et al. 1997) and in functioning as testimonials to the biodiversity present in a region (Bruner et al. 2001).

The state of São Paulo is a Brazilian region of extreme contrasts, harboring both heavily human-impacted areas and others that are well preserved, remain intact by human action, and support an exuberant biodiversity. Prominent among the preserved areas is the Juréia-Itatins Ecological Station (JIES), which was established by Brazilian federal law # 5,649/1987. The station comprises around 80,000 ha of preserved Atlantic Forest (Marques and Duleba 2004), which is currently conserved in a mosaic of conservation units (São Paulo 2006). In contrast, Cubatão is considered one of the most polluted regions in the world; in recent years, it has become the most important Brazilian industrial center, comprising 23 industrial complexes, 111 factories, and more than 300 pollution sources (Pinheiro et al. 2012). Gutberlet (1996) indicated that, since the 1960s, industrial pollutants have been the main cause of environmental problems in the Cubatão River and mangroves in this municipality, exemplified by blind fish with mottled skins and off-flavors.

Toxic effluents usually jeopardize the quality of water and sediments but, on the other hand, may also be a source of organic and inorganic substances that are used in biogeochemical cycles (Burton and Macpherson 1995; Burton et al. 2001) and food chains (Alongi 1998). Lima (2000) mentioned that many substances are potent environmental contaminants that generate a variety of physiological and behavioral responses in the biota. These contaminants may have deleterious effects on the ecosystem, alter the composition of populations (Eisler 2010a), or pose other subtle or unknown hazards (Monserrat et al. 2007). Metals are harmful inorganic pollutants in aquatic environments when their concentrations are higher than expected or tolerated. They can accumulate in water, sediment, or organic tissues through physicochemical or biological processes (Klerks and Weis 1987; Rainbow 2007; Eisler 2010b). Therefore, biological responses can be used to evaluate environmental changes (Buss et al. 2003) and thus provide useful information for actions to promote efficient monitoring and maintenance of environmental integrity.

Although few aquatic organisms are completely suitable as biomonitors (Miserendino and Pizzolón 2001), the sessile macroinvertebrates are highly important in assessing the conservation status of an ecosystem (Esteves 1988), especially for evaluations of water and edaphic quality (Junqueira et al. 2000; Monserrat et al. 2007). Benthic macroinvertebrates also receive particular attention because they can reflect the pollution history in an environment (Marvan 1979).

The "uçá"-crab (*Ucides cordatus*) has potential for use as a bioindicator of metals in mangrove areas (Pinheiro et al. 2012), based on its relatively greater longevity (Pinheiro et al. 2005), herbivorous diet (Geraldes and Calventi 1983; Nordhaus et al. 2006), and sediment bioturbation from burrow building (Nordhaus et al. 2009). This species is very important for the mangrove trophic web in the western Atlantic, as it is widely consumed by fish, birds, and mammals (Pinheiro et al. 2005; Wunderlich et al. 2008; Pinheiro and Fiscarelli 2009). It is an economically important fishery resource in Brazil and is extensively exploited along the entire coast, mainly in the North and Northeastern regions (Glaser and Diele 2004), where it is consumed locally and is also a source of income for traditional communities (Fiscarelli and Pinheiro 2002).

Some biomarkers (e.g., cytological, genetic, biochemical, or molecular) have been successfully employed to detect sublethal and deleterious effects on animal populations (Monserrat et al. 2007), quantifying the environmental stress and the effects caused by pollutants (Bayne 1986; Moore et al. 1986; Dorigan and Harrison 1987; Adam et al. 2010). Among these markers, the micronuclei assay quantifies the frequency of micronucleated cells (MNs) and has been used as a biomarker in the detection of sublethal genetic damage (Burgeot et al. 1995; Adam et al. 2010; Polard et al. 2011) from exposure to mutagenic genotoxic, aneugenic, and/or clastogenic substances (Countryman and Heddle 1976). It is also a potent biomarker for genetic damage in animals and is widely used in invertebrates (Scarpato et al. 1990; Wrisberg et al. 1992;

Hagger et al. 2009; Nudi et al. 2010; Jose et al. 2011) and vertebrates (such as fish: Nwani et al. 2010; Ahmed et al. 2011; Pavlica et al. 2011; amphibians: Jaylet et al. 1996; Marques et al. 2009; Yin et al. 2009; reptiles: Capriglione et al. 2011; birds: Quirós et al. 2008; Skarphedinsdottir et al. 2010; and mammals and humans: Sánchez-chardi et al. 2010; Elhajouji 2010).

An assessment of the basal frequency of micronucleated cells in *U. cordatus* living in a preserved ecological station is a matter of priority, especially at the Juréia-Itatins Ecological Station. Such an assessment is a rapid and inexpensive way to provide accurate data to aid in assessing environmental quality in Brazilian mangroves. It will also be very useful for Brazilian governmental agencies, since it will allow improvements in the national management plan proposed for this species (Brasil 2011).

The present study aimed to assess the conservation status of the uçá-crab (*U. cordatus*) by micronuclei assay (genotoxic effects) and the environmental quality of mangrove areas on the southeastern Brazilian coast (Juréia-Itatins and Cubatão), with two contrasting environmental situations, by measuring the concentrations of five metals (Cu, Cd, Cr, Pb, and Hg). These metals are the most often recommended by environmental agencies for assessment and are likely to be present in estuarine water and mangrove sediments.

Material and methods

Field sampling

Mangrove areas and capture of uçá-crab (U. cordatus)

Two mangrove areas were designated for the assessment of *U. cordatus* (Fig. 1): (1) *Juréia-Itatins* (24°26'03" S–47°05'03" W), on 5 February 2007, near the Una River mouth, within the JIES, and (2) *Cubatão* (23°52'50" S–46°22'25" W), on 20 May 2007, near the COSIPA/USIMINAS steel company, one of the most important factories in the Cubatão industrial center.

According to the classification of Köppen (see Rolim et al. 2007), Cubatão and Juréia have the same climate (Cfa), subtropical hot and humid, with the warmest month≥22 °C, coldest month<18 °C, and total rainfall in the driest month≥30 mm. Cubatão has one of the highest precipitation indexes (1,443 mm) in the state, with a dry period from April to September and a wet period from October to March (Mesquita 2011). Juréia-Itatins also has a high precipitation index (1,300 to 4,700 mm), considered by Narvaes et al. (2009) one of the rainiest coastal areas in the state, because of the proximity of the mountain massif to the sea (Galvani et al. 2012); with 2,278 mm mean annual precipitation, a rainy period from January to March (959.2 mm) and a drier period from July to August (276.3 mm) (Tarifa 2004). Both estuaries have semidiurnal tides (Por et al. 1984); the tides are recorded at the Port of Santos, located 15 and 95 km from Cubatão and Juréia-Itatins, respectively. The tidal range in Juréia-Itatins is 0.1 to 1.5 m, with very strong tidal currents and saltwater influence extending 15 km upstream (Por et al. 1984). Cubatão is located far inside the estuarine complex of Santos-São Vicente but still has a tidal range from 0.3 to 1.2 m and high salinities (27.6 to 35.0), suggesting a small continental inflow of freshwater (Belém et al. 2007). A remarkable difference between these mangrove areas is their distance from the sea, with a long sinuous route in the Cubatão area (21.8 km) and a short distance in the Juréia-Itatins area (1.8 km).

U. cordatus specimens were captured by the "braceamento" method (crab-catchers insert their arm into the burrow, as described by Pinheiro and Fiscarelli 2001). In each mangrove, ten adult male specimens of legal size (60 mm carapace width; Brasil 2003) and intermolt stage (see Pinheiro and Fiscarelli 2001) were caught. The crabs were placed in insulated boxes with brackish water and bags of ice in order to slow their metabolism. In the laboratory, they were cleaned of mud, measured with 0.05 mm precision calipers (carapace width (CW)) and weighed with a 0.01 g precision balance (wet weight (WW)). Then, the crabs were transferred to aerated aquaria with brackish water from the collection site, where they were kept until their hemolymph was sampled.

Sampling of water and sediment

For a better representation of each mangrove, samples of water were obtained in the same areas where the crabs were captured, at four locations, one of them from a boat in the estuary and the others obtained inside the burrows of *U. cordatus* at 10, 20, and

30 m from the riverbank. Water samples were obtained by suction with a silicon hose (first decontaminated with 10 % HNO_3, second with 10 % acid Extran®, and finally with deionized water) and stored in similarly decontaminated polyethylene flasks.

Sediment samples from mangroves were also collected in the same areas, at points 5 m from the riverbank, to provide a depth profile (four strata: surface and three subsequent strata, measured every 15 cm, to a total depth of 45 cm), each with three replicates. The effect of the sediment stratification on retention of metals was evaluated, considering that adults of *U. cordatus* are efficient scavengers and their galleries penetrate the sediment to a depth of 1 m or more (Pinheiro and Fiscarelli 2001). Sediment samples were carefully collected with the use of nitrile gloves, sieved to remove roots, and stored in polyethylene flasks.

All flasks with samples (water and sediment) were labeled and transported to the laboratory in insulated boxes with bags of ice and transferred to a refrigerator (5 °C) until the samples were analyzed, always within 30 days after collection.

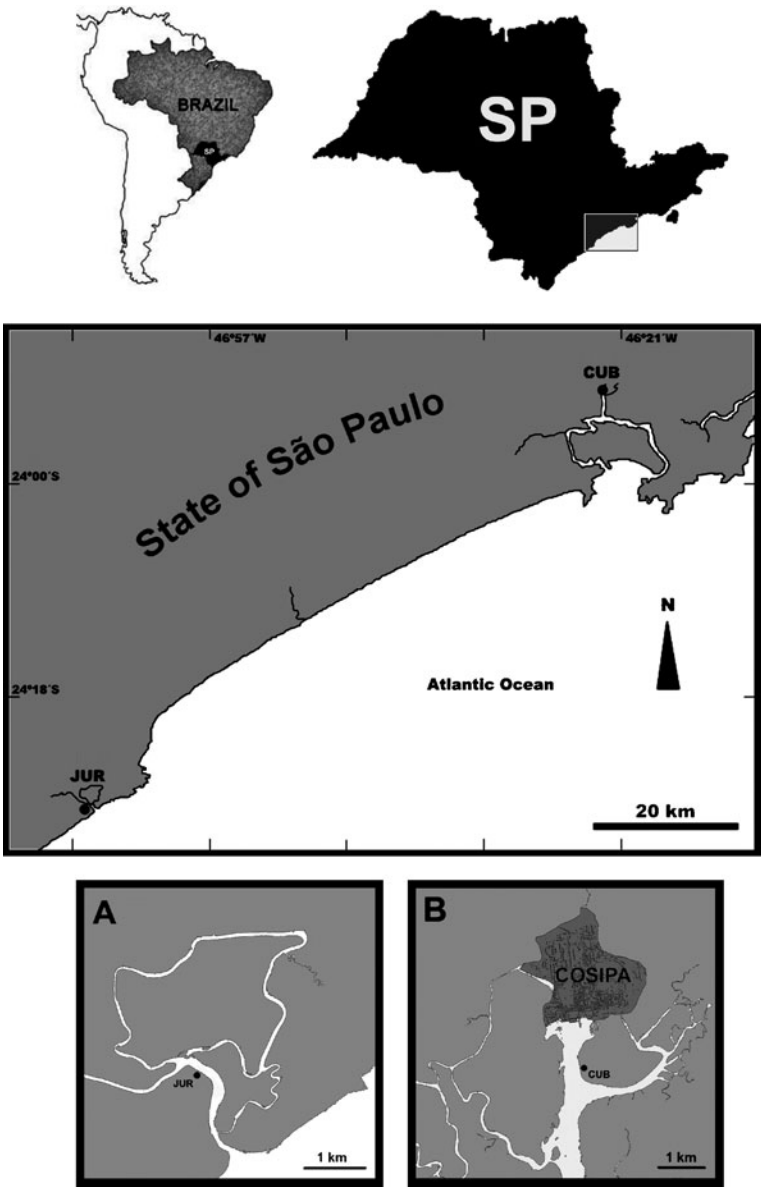

Fig. 1 Location of the mangrove areas sampled in the state of São Paulo, Brazil. **a** Juréia (JUR), the Juréia-Itatins Ecological Station (Environmental Conservation Unit); and **b** Cubatão (CUB), near COSIPA (Paulista Steel Company) in the Cubatão Industrial Center

Laboratory analysis

Micronuclei assay of U. cordatus

A sample of hemolymph (1 mL) from each crab was extracted with a hypodermic syringe with a 21-gauge needle (to avoid damage to the hemocytes, according to Nudi et al. 2010), positioned in the articulation membrane between the carpus and propodus of the cheliped. Each hemolymph sample resulted in three slides per crab, using the method developed by Scarpato et al. (1990) and modified for *U. cordatus* by Nudi et al. (2010). The slides were air-dried at room temperature, fixed with Carnoy solution (methanol/acetic acid 3:1), and again air-dried (20 min each). Slides were stained (20 min) with 2 % Giemsa in phosphate buffer pH 6.8 ($Na_2HPO_4+KH_2PO_4$) and then cleaned and washed with deionized water. The slides were air-dried and cover-slipped with Entellan® (Merck®). Each slide was carefully observed under a Zeiss® binocular microscope (1,000×), coupled to an AxioVision LE® (Zeiss) computer analysis system, to quantify the number of micronucleated cells per 1,000 analyzed (MN‰), based on three slides/crab.

Analysis of metals

In order to include the metals highlighted in recent reports issued by the environmental agency of the state of São Paulo (Cetesb 2001, 2007, 2009), the top five metal contaminants (Cu, Cd, Cr, Pb, and Hg) were quantified in samples of water and sediment. These analyses were executed by the Toxicology Assistance Center (Ceatox), located at IB/Unesp Botucatu, using an atomic spectrophotometer (GBC-932 AA), according to Basset et al. (1981) and Athanasopoulos (1994).

Data procedures and statistical analysis

Data were entered in spreadsheets, and the statistical analyses were conducted using 'R' Version 2.5.0 (Ihaka and Gentleman 1996). The frequency of micronucleated cells in *U. cordatus*, as well as the number of micronucleated cells per 1,000 cells evaluated (MN‰) were obtained from ten specimens from each location (n=10/mangrove). These data were used to calculate the normality by the Shapiro-Wilk test (SW) for each mangrove area, in relation to each metal concentration. Data with significant normal distribution were compared by Student's t test, otherwise Kruskal-Wallis was used to compare in this case the medians (Zar 1999), considering a statistical significance level of 5 %.

The measured concentrations of metals in water were compared with reference values for brackish water (minimum human impact or "class 1" according to Conama law # 357/2005 - Conama 2005). This Brazilian law does not provide the reference values for the assessment of estuarine sediments (Oliveira et al. 2007). Therefore, the results were compared with those provided by Environment Canada (1999) and Hortellani et al. (2008), according to two categories: (1) *Threshold effect level* (*TEL*), the concentration below which adverse biological effects are rarely observed (<10 %), and (2) *Probable Effect Level* (*PEL*), the concentration above which adverse biological effects are frequently observed.

Results

The mangrove areas differed in relation to water salinity ($p<0.05$), with the higher mean recorded at Juréia-Itatins (Table 1); the means of other environmental parameters were similar, confirming their homogeneity ($p>0.05$). The Juréia-Itatins mangrove was more flooded than the Cubatão mangrove ($p<0.05$), but the arboreal structure was similar (dominance of *Rhizophora mangle*>68 %), with different biometry of the trees (taller at Juréia-Itatins and thicker trunks at Cubatão).

The biometry of the crabs (CW and WW) did not differ statistically as a function of size (CW; $t=-0.44$; $p=0.67$) or weight (WW; $t=-1.73$; $p=0.10$) when the animals were compared between Juréia-Itatins (CW, 78.7 to 87.7 mm, 83.7±3.1 mm; and WW, 226.6 to 325.7 g, 276.4±33.6 g) and Cubatão (CW, 78.3 to 87.9 mm, 83.1±3.3 mm; and WW, 201.1 to 295.4 g, 249.1±37.0 g).

The numbers of normal and MNs, as well as their frequency (percent) in the specimens analyzed in both mangrove areas are presented in Table 2. In Juréia-Itatins, the crabs had a total of 59 micronucleated cells in a total of 30,000 analyzed (10 specimens × 3 slides × 1,000 cells). The frequency of micronucleated cells ranged from 0.03 to 0.37 %. In Cubatão, 156 micronucleated cells were found in a total of 30,000 analyzed cells. The frequency of micronucleated cells at this location ranged from 0.23 to 0.87 %.

Table 1 Means and standard deviations of pH and salinity of water inside burrows of *U. cordatus*, edaphic parameters, predominant mangrove tree species

Study area characteristics		Cubatão			Juréia-Itatins			t
Water properties	pH	6.9 (±0.1)			6.9 (±0.1)			0.37 ns
	Salinity (ppm)	26.4 (±2.0)			30.6 (±3.6)			−3.49*
Sediment properties	Grain size (φ)	5.2			5.4			−0.81 ns
	Sediment classification	Medium silt			Medium silt			–
	Organic matter (g/dm^3)	108.6 (±23.9)			96.2 (±39.2)			0.81 ns
Vegetation aspects	Dominance (%)	*A. schaueriana* 31.3	*L. racemosa* 0.7	*R. mangle* 68.0	*A. schaueriana* 5.3	*L. racemosa* 8.7	*R. mangle* 86.0	–
	Height (m)	8.3 (±1.9)			6.8 (±2.0)			6.58*
	DBH (cm)	10.8 (±4.5)			12.6 (±5.6)			−3.20*
Mangrove flooding	Height of *Bostrychietum* (cm)	32.9 (±8.8)			46.6 (±16.6)			−8.83*

DBH (tree diameter at breast height) and mangrove flooding (based on height range of algae of the genus *Bostrychietum* spp. on tree trunks) (t=test t; *ns*=not significant to 5 %)

*p<0.05

The frequency of micronucleated cells/crab in each mangrove was converted to MN‰. The data normality was confirmed by the Shapiro-Wilk test (Juréia-Itatins, SW=0.96; p=0.80; and Cubatão, SW=0.96; p=0.86). In Cubatão the mean of MN‰ (5.2±1.8 MN‰) was 2.6 times higher than that recorded for Juréia-Itatins (2.0±1.0 MN‰). This difference was statistically significant (t=4.98; p=0.0001) (Fig. 2).

The water samples had detectable Pb concentrations. However, the other metals had concentrations (micrograms per milliliter) below detection limits, for both mangroves (Cu and Cr, <0.05; Cd, <0.01; and Hg, <0.0001). At Juréia-Itatins, the Pb concentration was lower than 0.05 μg/mL in the estuary and at 10 m from the riverbank. The Pb concentration was 0.12 and 0.28 μg/mL, respectively, at 20 and 30 m from the

Table 2 Number of normal and micronucleated cells (MN) with their frequency (%) in specimens of *U. cordatus* analyzed in mangroves from Cubatão and Juréia-Itatins, state of São Paulo, Brazil

Specimen analyzed	Juréia-Itatins			Cubatão		
	Normal cells	MN cells	Frequency (%)	Normal cells	MN cells	Frequency (%)
1	2,992	8	0.0027	2,993	7	0.0023
2	2,989	11	0.0037	2,988	12	0.0040
3	2,994	6	0.0020	2,987	13	0.0043
4	2,990	10	0.0033	2,985	15	0.0050
5	2,997	3	0.0010	2,988	12	0.0040
6	2,999	1	0.0030	2,981	19	0.6300
7	2,996	4	0.0013	2,974	26	0.8700
8	2,996	4	0.0013	2,982	18	0.6000
9	2,994	6	0.0020	2,986	14	0.4700
10	2,994	6	0.0020	2,980	20	0.6700
Total	29,941	59	0.0020	29,844	156	0.0052

riverbank. In Cubatão, the Pb concentrations varied only slightly among the water samples (0.12–0.19 μg/mL). However, based on the normality of Pb concentration values (Shapiro-Wilk, $p>0.05$), the mean concentrations of Pb in these mangroves did not differ significantly ($t=0.73$; $p=0.51$).

The concentrations of metals in the different sediment strata in both mangroves are presented in Table 3. Mercury was the only metal not detected (<0.001 μg/g). The other metals did not show normal distributions (Shapiro-Wilk, $p<0.05$). They also showed higher concentrations at Cubatão than at Juréia-Itatins (Kruskal-Wallis, 5.81<KW<16.34; $P<0.015$; Fig. 3). The concentrations of Cd, Cu, Cr, and Pb did not differ among sediment strata (0.40<KW<1.64; $p>0.41$), in both mangroves.

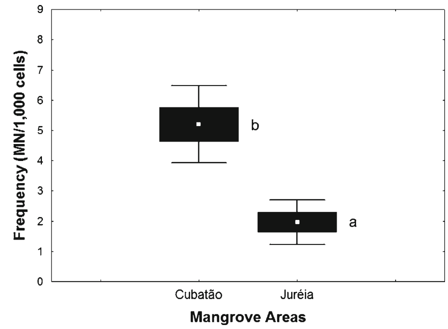

Fig. 2 Frequency of micronucleated cells (MNF) on slides of hemolymph from the crab *U. cordatus* ($n=10$/area) caught in mangrove areas in Cubatão and Juréia (Juréia-Itatins Ecological Station), in the state of São Paulo, Brazil. *White dot*=mean; *black box*=mean±standard error; *whiskers*=mean±5 % confidence interval; and *letters*=means with different letters are statistically different ($p<0.05$)

Discussion

The mobility and bioavailability of metals in the environment are dependent on their total concentration and the influence of different physical–chemical parameters, among them the pH and salinity of water (Hatje et al. 2003). However, Atkinson et al. (2007) found higher rates of metal release (and sequestration) when the water is alkaline or the sediment is disturbed than with increases of oxygen or salinity in the water. In the present study, water from both mangrove areas had similar pH; the higher salinity in Juréia-Itatins was not accompanied by an increase in metal concentrations. Britto et al. (2006) mentioned that precipitation can also influence the environmental quality, and this effect is greater in more-impacted estuarine systems due to leaching from adjacent terrestrial areas by rain (Dunn et al. 2007). Both mangrove areas in this study have similar annual precipitation indexes, indicating that the seasonal influence of this parameter when the samples were obtained is likely to be low and confirming a different contamination level between an area with better environmental quality (Juréia-Itatins) and a more polluted one (Cubatão).

With the exception of Pb, the metal concentrations in water were below the reference values established by law (Table 4), independently of the mangrove studied. Higher concentrations of Pb were expected in water samples from the Cubatão mangrove, because of the local pollution history (Cetesb 2001), although the levels recorded were below that established for "class 1" brackish water (minimum human impact). These data contrast with those from the Juréia-Itatins mangrove, which contained half the amount of lead, in agreement with the better water quality expected for

Table 3 Concentration of metals (mean±standard error, in micrograms per gram), in the sediment at four depth strata, in mangroves from Cubatão and Juréia-Itatins, state of São Paulo, Brazil

Mangrove areas	Samples	Copper (Cu)	Cadmium (Cd)	Chromium (Cr)	Lead (Pb)
Juréia	Surface	1.92±0.12	0.053±0.003	3.17±0.17	4.56±0.14
	15 cm	1.30±0.17	0.046±0.003	3.07±0.61	3.18±0.25
	30 cm	1.06±0.12	0.043±0.003	2.14±0.17	2.78±0.53
	45 cm	1.72±0.45	0.056±0.007	4.37±1.33	3.97±0.85
Cubatão	Surface	3.82±0.37	0.064±0.009	6.39±0.74	5.33±0.53
	15 cm	4.05±0.22	0.077±0.013	6.91±0.40	6.18±0.53
	30 cm	3.78±0.21	0.063±0.009	7.47±1.25	5.41±0.21
	45 cm	2.81±0.49	0.057±0.009	5.24±0.97	3.86±0.95

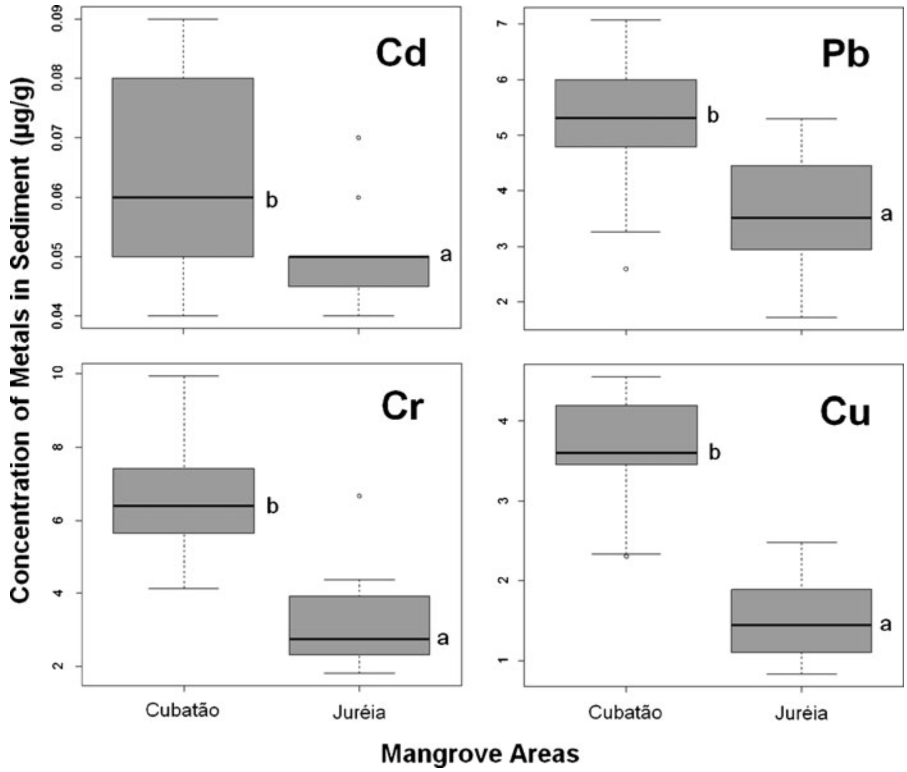

Fig. 3 Concentration of metals (microgram per gram) measured in sediment samples from mangrove areas in Cubatão and Juréia-Itatins, state of São Paulo, Brazil. *Line inside box*=median; *box*=quartile; *whiskers*=amplitude; and *letters* =medians with different letters are statistically different ($p<0.05$)

this ecological station. Lower concentrations (<PEL) of metals in sediment were also recorded in both mangrove areas (Table 4), indicating a probable effect level (PEL) on the aquatic organisms (Environment Canada 1999).

According to Kennish (1997), contamination by Pb in surface water and sediment in most estuaries and adjacent environments results from the wide use of gasoline, ink pigments, and electric batteries. Quiterio (2000) assayed the atmospheric component of this pollutant as metallic particles or oxides, which were frequently observed in the recent past in Cubatão factories (Alonso 1996). However, Cetesb (2001) reported a lower level of Pb in the Santos-São Vicente estuary than was found in this study.

Copper has a wide variety of applications, as the metal (e.g., electric wires), oxide (e.g., chemical catalyst), or sulfate (e.g., algicide). This metal has been previously

Table 4 Reference values of threshold effect level (TEL) and probable effect level (PEL) for concentrations of metals in sediment and brackish-water Class 1 (with minimum human impact)

Metal	Sediment[a] (µg/g=mg/kg)		Brackish water[b] (µg/mL=mg/L)
	TEL	PEL	
Cadmium (Cd)	0.67	4.20	0.005
Lead (Pb)	30.0	110.0	0.010
Copper (Cu)	19.0	110.0	0.005
Chromium (Cr)	52.0	160.00	0.050
Mercury (Hg)	0.13	0.70	0.0002

[a] Hortellani et al. (2008)

[b] Brazilian law (Conama 2005)

detected with other trace metals in effluents of some Cubatão industries (refinery, metal, and paper/pulp), bulk liquid terminals, or liquid waste from open dumps, sanitary landfills and sewage (Lamparelli et al. 2001). The enormous human impact at Cubatão was confirmed in the sediment samples analyzed here, which showed almost twice the concentration recorded at the Ecological Station of Juréia-Itatins, where human activities are strictly limited.

Chromium was formerly used to prevent corrosion in cooling systems of Cubatão factories (Tessler et al. 2006; Fukumoto 2007), resulting in higher concentrations in the Cubatão River watershed, although these levels were below the detection level according to Cetesb (2001). The reduction in Cr contamination is probably the result of a rigorous pollution-control program established in this area since 1984 (Cetesb 1990), where the replacement of cooling systems was proposed (Lamparelli et al. 2001).

Cadmium was also very frequently found during environmental monitoring by Cetesb (2001) in this area, mainly in effluents from fertilizer and petrochemical factories at Cubatão, and also released into the atmosphere, as shown by Gutberlet (1996) who evaluated mosses (*Sphagnum*). The higher concentrations of Pb, Cu, Cr, and Cd recorded at Cubatão than at Juréia-Itatins could well be explained by these human activities in past years in its estuarine system.

According to Scarpato et al. (1990) and Fossi et al. (2000), the basal average micronuclei frequency detected for *Mytilus galloprovincialis* (bivalve) and *Carcinus aestuarii* (crab) was <4 MN‰. The micronuclei frequency is species-specific. For *U. cordatus* captured in the Juréia-Itatins ecological station, the frequency was around 2 MN‰, whereas at Cubatão the frequency was 5.2 MN‰, certainly because this is the most polluted mangrove area in the state of São Paulo. Nudi et al. (2010) indicated that *U. cordatus* is a highly genetically sensitive species in terms of aquatic environmental monitoring. The micronuclei data observed here reinforce this hypothesis; although the metal concentrations found at Cubatão were not as high as expected (except for Pb), some pollutants could have a genetic impact (Bijlsma and Loeschcke 2012). Genotoxicity tests are highly sensitive to detect genomic damage, even when pollutants are present in smaller concentrations (Monserrat et al. 2007; Sponchiado et al. 2011). Nudi et al. (2010) mentioned that the uçá-crab is not efficient as a PAH biomarker under field conditions, although it is known that organic pollutants can cause genetic impacts in many species. Although recent studies revealed significant genetic changes caused by metals during exposure at different concentrations, including lead (Celik et al. 2005; Piao et al. 2007; Alghazal et al. 2008; Tapisso et al. 2009; García-Lestón et al. 2010), copper (Franke et al. 2006; Serment-Guerrero et al. 2011), chromium (Papageorgiou et al. 2007; 2008), and cadmium (Seoane and Dulout 2001; Bertin and Averbeck 2006; Ahmed et al. 2010; Otomo and Reinecke 2010).

In the Cubatão mangroves, metal contamination was detected in concentrations below TEL and PEL levels. However, analyses of a larger number of specimens will be necessary to adequately evaluate the association between the Pb levels and the MN‰ results for *U. cordatus*. The data obtained indicate a strong possibility of genetic depletion in this species, with a high frequency of MNs in the specimens from the Cubatão mangroves. The genetic impact may have occurred as a result of the high concentrations of Pb in synergy with other pollutants in Cubatão, in view of the contamination history of this municipality (Gutberlet 1996). Among the recorded pollutants are oxides (sulfur and nitrogen), carbon (monoxide and dioxide), methane, hydrocarbons, organochlorine compounds, chlorinated phenols, fluorides, aldehydes, acids, and particulate material dissipated by air (Cetesb 2001, 2007). More recently, Duarte et al. (2012) conducted a broad evaluation of metal concentrations in mangrove sediments in the state of São Paulo, and confirmed a significant association between the mercury concentration and the number of micronuclei in *U. cordatus*. Exemplifying the effect of the environmental contamination, one specimen of *U. cordatus* with a malformed cheliped was collected in São Vicente by Pinheiro and Toledo (2010), in the same estuary of Cubatão. This specimen showed 11.5±2.0 MN‰, which was 2.3 times higher than that recorded for this species in the present study.

Besides uçá-crab catching, several other estuarine organisms are harvested in the Cubatão mangroves (e.g., fish, swimming crabs, bivalves, etc.) as a traditional fishery activity by traditional communities (46 % of the families living in Cubatão Municipality, according to Santos-Filho et al. 1991 and Gutberlet 1996). However, previous studies have demonstrated the inadvisability of consuming the local fish, particularly those from Cubatão (Lamparelli et al. 2001), because of environmental contamination and deleterious genetic effects. The impacted region (Cubatão and its surroundings) includes part of a protected ecological area (Atlantic Rain Forest), as well as several traditional human communities that depend on the diverse aquatic fauna for their subsistence. Therefore, the data revealed in this study indicate the urgent need for better management of pollutants in the region.

More frequent environmental monitoring will be needed to prevent these adverse effects on the local biota, and the time and cost of monitoring can be minimized by the use of easier and less-costly techniques such as the micronucleus test. The present study demonstrated the success of this technique applied to *U. cordatus*, supporting its usefulness for monitoring mangrove areas, particularly in regions that have experienced chemical or microbiological impacts. This genotoxic technique can be used as an additional tool to provide useful information to support decisions regarding mangrove conservation.

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