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Paraquat Contamination in Surface Waters of a Rural Stream in the Mountain Region in the State of Rio De Janeiro Southeastern Brazil

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Abstract

Paraquat is an herbicide widely applied to agriculture in Brazil. As it has human toxic properties, it is of great interest for public health to evaluate water contamination, especially when the local population consumes water from aquifer springs near crops. Paraquat concentrations were assessed in surface waters of a stream that crosses a rural area of intensive agricultural production in the mountainous region of Rio de Janeiro. Water samples were collected at 7 sampling sites (n=84) during 1 year, and paraquat concentrations were determined by ELISA. Residues were found in 62.5% of the samples. The sampling point located closest to the plantation area was the most frequently contaminated (average 0.075µg/L; maximum 0.279µg/L). The local rainfall regime seems to be an important predictor of paraquat contamination according to Spearman's correlation coefficient (R=0.7053; p=0.0128). The results suggest that paraquat residues in water represent a distinct threat to human and environmental health, especially after rainy periods.

Keywords: Paraquat; Stream contamination; ELISA; Public health; Environmental safety

Abbreviations: ELISA-Enzyme-Linked Immunosorbent Assay; K_{ow}-Octanol/Water Partition Coefficient; K_{oc}-Organic Carbon Adsorption Coefficient; DT_{50}-Half-life; K_H-Henry's Law Constant; GUS-Groundwater Ubiquity Score

Introduction

Brazil is known as the world´s top pesticide user. Data from the past decade show a 50% increase in pesticide consumption in Brazil, and in 2011, pesticide use consisted of approximately 852.8 million liters, corresponding to US$ 8.5 billion in pesticide sales [1]. Potential harmful effects in pesticide-exposed populations have increasingly come to the attention of the scientific community. Most studies regarding Public Health focus mainly on direct pesticide exposure, despite the fact that indirect exposure is extremely relevant. Aquifers can expose communities to several environmental pollutants, given the compartmental mobility of these substances due to their physicochemical properties [2,3]. Most substances are able to actively migrate between environmental compartments, leading to differential contamination effects. Among rural populations, pesticide exposure occurs during agricultural duties. In addition, climate can also directly interfere with human exposure since warmth, wind and rainfall characteristics can lead to differences in exposure profiles between populations [4,5]. Pesticide residues are commonly assessed in drinking water [6], and maximum permissible limits for pesticide contamination are internationally established [7,8]. However, it is essential to consider that rural populations involved in intensive agricultural production areas are directly exposed to many sources of contaminated water compartments. Most of the populations living in developing countries such as Brazil do not have access to water or sewage distribution systems. Consequently, rural communities depend entirely on groundwater for domestic and agricultural purposes, eventually ingesting contaminated water from water tables that spring near crop fields that have been sprayed with pesticides.

Communities with certain agricultural characteristics in the mountainous region of Rio de Janeiro are leading fruit and vegetable producers. However, an enormous amount of pesticide use in this area has led to the exposure of these populations to up to 56 kg of pesticide per worker per year [9].

In contrast with developed countries that run regular pesticide assessments of superficial and groundwater, in Brazil there is no monitoring of pesticides in aquifers unlike piped drinking water. Despite laws that limit worker exposure to pesticides, there is little action from the government to regulate either trade or proper use of these chemicals. Surface and groundwater contamination by pesticides have been reported in many studies conducted worldwide [10-15], including Brazil [16-22]. In this context, the present study was aimed at conducting a screening assessment for paraquat in the Sao Lourenço stream, located in the mountainous region of Rio de Janeiro, because this herbicide is widely used by this community. Paraquat is a well-known and classic pulmonary redox-active toxicant [23] and its physical-chemical characteristics are listed in table 1.

This herbicide has been banned in the European Union since 2007 and its use by licensed farmers is restricted in the US. The Brazilian Health Ministry and the Environmental Agency (CONAMA, in Portuguese) define limits and screening criteria for pesticides in drinking, surface and groundwater, however, there is no established limit for paraquat stipulated in these guidelines [26,27]. Thus, this knowledge is expected to contribute to improving environmental protection designs within Brazilian legislation.

Materials and Methods

Study site

The São Lourenço stream has its headwaterin the rain forest at Caledonia Peak in Nova Friburgo, one of the highest points in amountainrangelocated parallel to the Atlantic Ocean in Brazil, called Serra do Mar. The São Lourenço River flows into the Paraiba do Sul river, one of the most important in the country, and together account for 200 km bodies of water [28]. The São Lourenço stream crosses the São Lourenço village, whichbasically consists offarming families. The region's topography is composed of colluvial slopesand the local cropsreach the waterbed. Paraquatspraying in this region is done manually by means of knapsack sprayers, all year round due to the good climate of the region, being applied as soon as weed stake root or when farmers wish to clean up the fields.

Surface water sampling, annual rainfall data and paraquat determinations

The upper São Lourenço streammerges with several other streams that cross villages with the same agricultural characteristics and pesticide use as São Lourenço. When water volume increases, the São Lourenço stream becomes the Rio Grande (Big River). Surface water samples were collected from October 2011 to December 2012 in 1 L PET bottles. In total, 80 surface water samples were collected from seven different sampling sites along the stream, starting at the base of Caledonia Peakin the rainforest near the Sao Lourenço source to three points of the Rio Grande (Figure 1). The last sampling site represents the entire microregion, as it receives other effluent from different watersheds that cross neighboring regions with the same agricultural production characteristics.

All samples were maintained at 4°C between sampling, transportation and laboratorial analysis. The rainfall regime (mm) data of the region were obtained from the Institute of Meteorology (INMET). Paraquat concentrations in surface water were measured using commercially available ELISA kits (EnviroLogix and Abnova) in accordance with the manufacturer's protocols. In brief, ELISA for paraquat is based on the competition between paraquat in the water sample and paraquat-horseradish peroxidase conjugate, for binding to the antibody against paraquat, coated onto microwells. The addition of a chromogenic substrate then measures the bound enzymatic activity. After washing steps, the outcome of the competition is visualized with a color development step. As with all competitive immunoassays, the sample concentration is inversely proportional to the color development. The samples were analyzed in duplicate with no prior treatment and absorbance was determined on an Expert Plus microplate reader at λ= 450 nm.

Statistical analyses

Data are reported as average ± standard deviation (SD). In order to identify significant differences in concentration between the sites, a one-way analysis of variance (ANOVA) was

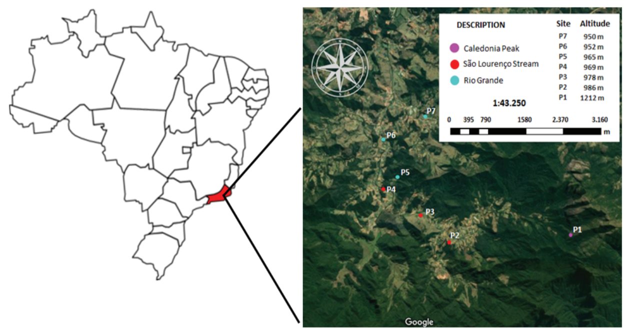

Figure 1: Geo-referenced image of the surface water sampling sites at the São Lourenço stream, Nova Friburgo, Rio de Janeiro, Brazil. Point 1 (P1) represents the control site near Caledonia Peak, P2-P7 are ascending sampling sites located at the upper stream flow. Source: Google maps.

Table 1: Paraquat physico-chemical characteristics at 20-25°C.

Vapor pressure, (mmHg)	Water solubility, mg L^{-1} at 20°C	Log K_{ow} $^{(1)}$	K_{oc} $^{(2)}$ cm^3 g^{-1}	DT_{50} $^{(3)}$ in soil, days	DT_{50} a hydrolyses, days	K_H $^{(4)}$ atm m^3 mol^{-1}	GUS $^{(5)}$
<1 × 10^{-5}	6.2 × 10^5	4.22	15473-1000000	1,000	-	1 × 10^{-9}	0.57

$^{(1)}K_{ow}$=Octanol/Water Partition Coefficient; $^{(2)}K_{oc}$=Organic Carbon Adsorption Coefficient; $^{(3)}DT_{50}$=Half-Life; $^{(4)}K_H$=Henry's Law Constant; $^{(5)}$ (GUS)-Calculated Groundwater Ubiquity Score [24,25].

applied. The monthly rainfall regime was statistically considered by non-parametric tests for either total monthly paraquat concentrations or single site paraquat concentrations, evaluating Spearman correlation coefficients. Unless stated otherwise, a two-tailed P-value <0.05 was considered statistically significant. All analyses were performed with the SPSS version 17 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 5.1 (GraphPad Software Inc., San Diego, CA, USA) software packages.

Results

Paraquat concentrations across seven sampling sites in the upper São Lourenço stream course

The limit of quantification for the technique was 0.02 µg L^{-1}. Paraquat was measurable in 62.5% of samples. A significant trend of increasing paraquat concentrations along the stream course was observed (p=0.008) (Figure 2). The last sampling site (P7), already in the Rio Grande, showed a higher frequency of contamination, with average of 0.075 µg L^{-1} (up to 0.279 µg L^{-1}). The number and the extension of crops increased exponentially along the sampling sites, explaining the increasing paraquat concentrations measured.

Correlation between monthly rainfall regime and paraquat concentrations

Annual measurements of monthly rainfall (mm) average between October 2011 and December 2012 was registered by the INMET via an automatic weather station in Nova Friburgo and is displayed in figure 3. São Lourenço has a pronounced rainy season during summer, from November to January, as it is a tropical region, located at low latitude in southeastern Brazil.

Spearman's correlation test indicated that paraquat concentrations in the evaluated surface waters correlated positively with monthly precipitation throughout one year (R=0.7053, p=0.0128) (Figure 4). Increasing positive correlations between monthly rainfall and paraquat concentrations in surface waters were observed at the upper stream course when stratified by sampling sites, except for the control site (P1), which showed an inverse correlation. From P4 onwards significant and moderate correlations were found, whereas the highest correlation was found at the last sampling site (Table 2).

Discussion

The data presented here in shows evidence of consistent, low, paraquat levels in surface waters of the São Lourenço stream, which crosses a region of intense agricultural production and pesticide application. The control site, near the São Lourenço stream source, located in a rainforest area up the Caledonia Mountain Peak, showed no paraquat contamination. The stream is joined by other streams and water volume increases through its upper course. Concomitantly, crops also increase exponentially, advancing through the colluvial slopes until the stream waterbed, explaining the increasing paraquat concentrations measured along the sampling sites.

Table 2: Spearman correlation coefficient (r) the width p-value of monthly rainfall regime (mm) and paraquat concentrations (µg L^{-1}) correlations at each sampling site of the São Lourenço stream.

Sampling site	r	P	Correlation strength**
P1	-0.470	0.240	moderate
P2	0.303	0.339	weak
P3	0.420	0.174	moderate
P4	0.609	0.035*	strong
P5	0.706	0.010*	strong
P6	0.296	0.351	weak
P7	0.771	0.030*	strong

*Statistically significative
***[29]*

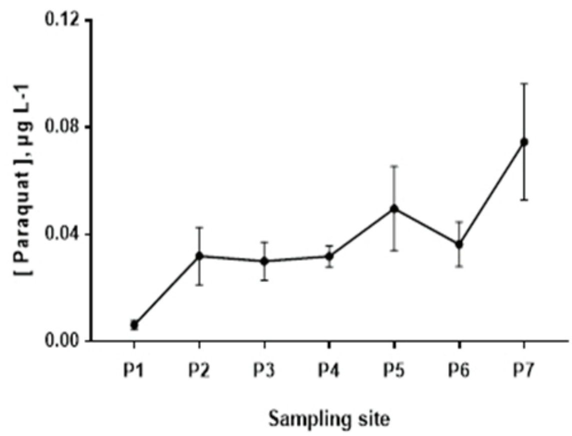

Figure 2: Annual average (from October 2011 to December 2012) of paraquat concentrations in surface water from seven sampling sites of the São Lourenco stream. Data are expressed as µg L^{-1} and depicted as average ± SD.

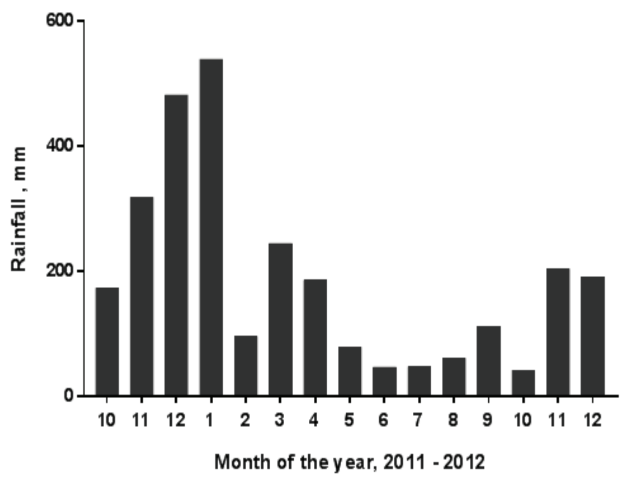

Figure 3: Monthly rainfall regime from October 2011 to December 2012 in Nova Friburgo, RJ. Source: INMET.

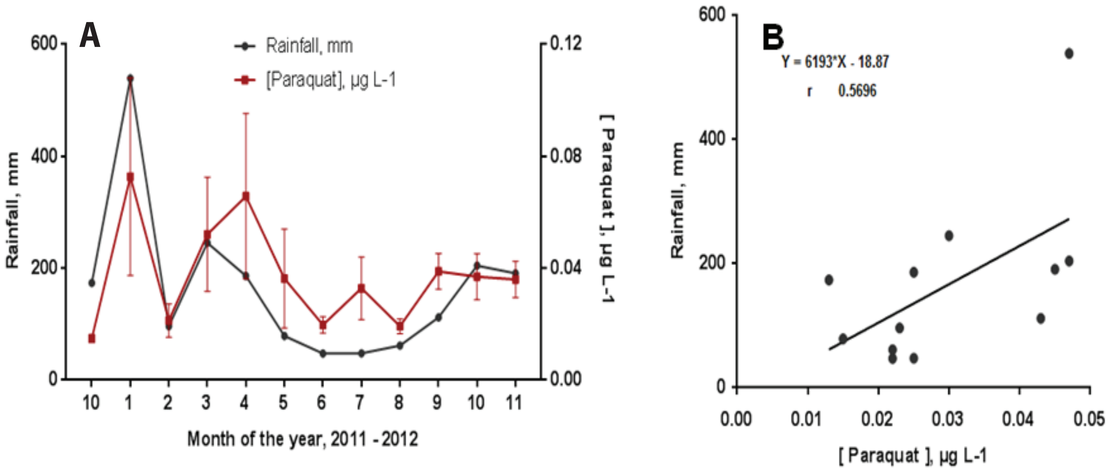

Figure 4: Rainfall (mm) versus paraquat concentrations (µg L^{-1}) from October 2011 to December 2012 in surface water samples of the São Lourençо stream. **(A)** The "Y" axis (left) represents monthly rainfall regime (mm), "y" axis (right) average paraquat concentrations (µg L^{-1}) and the "x" axis presents monthly measurements. **(B)** Scatter plot of the monthly rainfall (mm) and paraquat concentrations (µg L^{-1}), indicating the Spearman coefficient correlation and the linear equation.

During intense rainfall regimes, increased concentrations of dissolved organic matter are usually observed, as well as particulate matter, which carries runoff to the surface waters of water bodies [30,31]. Extreme weather conditions with high precipitation volumes can trigger landslides that may enhance pesticide mobilization to the surface water. For example, [32] reported pesticide mobilization to aquatic environments after torrential storms in Canada. On the other hand, low pesticide concentrations in water bodies are commonly observed during drought periods, which can be justified by the absence of surface runoff caused by rainfall [33,34].

Previous studies conducted in the same region revealed significant levels of anticholinesterase pesticides in surface waters of the São Lourenço stream [35]. Another study conducted in the Southeastern Brazilian region detected pesticide contamination in 70% of samples collected during five months, and the authors hypothesized that pesticides applied in agriculture could migrate and reach groundwater and surface water sources [36]. Other surveys performed in Northeastern Brazil assessing the main pesticides used in an agricultural area and identifying them in surface water and groundwater also support this suggestion [37].

Pesticides strongly sorbed in soil may travel and enter surface waters, while more water-soluble pesticides and those weakly sorbed in soil may be present in the groundwater solutions; reaching surface water as runoff [2,3,38]. Both mechanisms allow pesticide migration and allocation to Bodies of water. The annual rainfall regime average during the period evaluated in the present study was 188 mm, although this increased to above 250 mm during four months, suggested by the EPA as a volume with a potential to be a surface water contaminant [39,40]. With regard to paraquat physical-chemical properties, in addition to its high water solubility, the organic matter high affinity (Koc= 15-1000000) of this compound should restrict paraquat soil mobility. Theoretically, paraquat leaching is hampered through the formation of an organic matter-paraquat aggregate and, consequently, only a small fraction of this herbicide would be available to be flushed out towards the stream's surface water. Additionally, even if paraquat were to reach stream water, it would move to aquatic weeds and sediment, with consequent deposition onto the beds of surface water bodies [41]. However, other factors should be taken into account regarding pesticide mobilization. Based on the groundwater vulnerability assessment approach implemented by the U.S. Environmental Protection Agency (USEPA), Groundwater Ubiquity Score (GUS) and the method proposed by Goss, paraquat is classified as a possible groundwater contaminant [42-45]. Besides, due to high sediment-transportation, paraquat can be washed off eroded soil from colluvial slope crops and reach surface waters (42,46), especially during high precipitation periods. Data for paraquat persistence in tropical soils are still limited, and many environmental variables are involved in predicting inter-compartmental pesticide migration. However, investigations regarding environmental paraquat fate have proven that paraquat mobility depends on soil characteristics. For example, in Thailand, researchers reported desorption in sandy loam soils as well as paraquat detection in groundwater [47]. Another study in Malaysia using 14C-labeled paraquat demonstrated that its adsorption was increased in higher soil pH [48].

Paraquat levels detected in São Lourenço stream surface waters were below the maximum permissible levels, however, given the above, the extension of the local soil and sediment contamination are still a concern. High levels of paraquat in soil could also lead to groundwater contamination and may result in long-term effects on the water supply of the rural population. From a public health point of view, the adverse effects of pesticide pollution can be exacerbated due to lack of infrastructure and socioeconomic deficiency. The public water supply of this region is mainly obtained from undergrounded aquifers. Despite the fact that neither soil nor groundwater were analyzed for the presence of the evaluated herbicide, whether this contamination can be extended to water tables that spring around the region and supply the local population remains a concern. Although this study only evaluates paraquat among the multiple pesticides used in the survey area, the quantification of environmental pollutants is a valuable tool for the implementation of adequate regulations by the authorities. A systematic environmental risk assessment would further investigate paraquat levels in soil and would be able to predict potential hazards to human health more accurately. Furthermore, it is crucial to consider the risks that small streams bring to rivers that supply major cities. In this case, the Rio Grande, an important supplier of water to the city of Nova Friburgo, with a population of 184,786 inhabitants, is the recipient of stream waters originating the São Lourenço stream, [49]. Thus, paraquat and other toxic compounds should be further evaluated in different environmental compartments of this area, since they may pose toxic health risks to both the rural community and the general population.

Conclusion

This study provided an overview of the agricultural impact on surface waters of the São Lourenço stream. A significant trend of increasing paraquat concentrations along the stream course was observed and it was mainly related to the exponential increase in the number and extension of crops along the sampling sites. Moreover, paraquat concentrations in the surface waters were correlated positively with monthly precipitation throughout one year, and it was correlated with the enhanced pesticide mobilization from the crop fields to the surface water during intense rainfall regimes. It was demonstrated that paraquat, a widely used pesticide in this region and not-monitored in current Brazilian legislation, is able to reach surface waters and should be monitored by authorities in answer to public health concerns. In addition, data also indicated that paraquat concentrations in surface water were directly correlated with seasonality depending on the rainfall regime, which may be related to leaching and runoff. Although the levels found in the area were below the recommended limits, the results still indicate contamination of surface water by the local agricultural activities.

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Conflict of Interest

There are no conflicts of interest to declare.

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