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Solvatochromic Dyes Track a Homeopathic Preparation through Water-Stream Systems in a Program to Control Tick Infestation at a Wild Animal Rehabilitation Center in Brazil

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Abstract

Introduction In 2021, the area of CRAS (Centro de Reabilitação de Animais Selvagens – Wild Animal Rehabilitation Center), located in a state park in Campo Grande City, Mato Grosso do Sul, Brazil, was suffering from a tick infestation affecting wild animals that inhabit the area and humans that visited its trails. Following a formal technical–scientific cooperation agreement between IMASUL, an institute for the environment in Mato Grosso do Sul state, and SIGO Homeopatia, a formulated homeopathic complex (*Formula Parques Urbanos*, FPU) was designed and prepared specifically to treat the animals. This environmental intervention used specially designed slow-release water biodegradable devices. Tracking the FPU signal in water was necessary to monitor and manage the intervention.

Aims Our aims were (1) to evaluate, among six previously standardized solvatochromic dyes, which would serve as a marker for the homeopathic complex under study; and (2) to evaluate whether the chosen solvatochromic dye could map the propagation of the homeopathic complex activity throughout the stream system from water samples harvested at different locations over time.

Method Water samples were harvested from each point at different times, filtered, frozen, and sent to the laboratory, where they were prepared at 1cH potency for analysis using 30% ethanol as the vehicle. Solvatochromic dyes were used to analyze the samples since they alter their absorbance when in contact with homeopathic potencies. Of the six dyes tested, Coumarin 7 was found to be the most suitable for tracking the FPU complex. A static and average unidirectional magnetic field of 2,400 Gauss (240 mT), generated by a neodymium magnet, was applied to the samples immediately before reading.

Results There were significant differences in the delta absorbance of dyes when adding treated/potentized water samples, making it possible to map the propagation of the FPU signal throughout the park over time. The signals were identifiable at the

Keywords
- environmental homeopathy
- water
- solvatochromic dyes
- ecosystems
- magnetic field

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same point 1 minute and 32 days after the insertion of the device into the water. These signals were also identifiable after 75 minutes and 8 days at a point far from the insertion place.

Conclusion Coumarin 7 was the best marker for the homeopathic complex (FPU) used to treat the wild animals living in the park. The microplates/enzyme-linked immunosorbent assay reader method and the application of a magnetic field to samples were shown to be effective in tracing homeopathic signals by changes in dye absorbance ($p \leq 0.02$) in a real-life situation, with large volumes of water, involving many environmental variables, and over large distances.

Introduction

Insect and arachnid infestations have been a significant challenge around the world. Insecticides are being used to control plagues but with temporary results and a high cost to the ecosystem. Utilizing homeopathic products could be a more balanced and effective way to treat those infestations.[1,2]

In this context, the company SIGO Homeopatia, following an approach by IMASUL (Instituto do Meio Ambiente do Mato Grosso do Sul), an institute for the environment in Mato Grosso do Sul state, formulated a homeopathic complex to treat an infestation of ticks (*Amblyoma cajennensis*) that occurred in 2021 at CRAS (Centro de Reabilitação de Animais Selvagens), a center for rehabilitation of wild animals in Brazil. The study, using the formulated complex named *Formula Parques Urbanos* (FPU), was underpinned by a formal technical–scientific cooperation agreement between the two institutions.

The CRAS is located in the Prosa Park, Campo Grande city, Mato Grosso do Sul. It is linked to two other parks, the Powers' Park and the Indigenous Nation Park (PNI), comprising a single natural area in the urban context, whose extension is 2.7 km long. The city is close to one of the most important Brazilian biomes, the Pantanal. Thus, CRAS has been essential for animal well-being and local wildlife conservation. Recently, the first veterinary hospital dedicated exclusively to wild animals was built in this area.

In 2021, this tick infestation affected wild animals and humans (the park staff and visitors), culminating in the three parks' closure for school excursions, given the risk of spreading zoonosis. In the parks, there is a population of capybaras and tapirs who live in a free-roaming condition with free access to water and a special preference for a lake located at Indigenous Nation Park, where animals tend to stay immersed. These animals are the most susceptible to tick infestations.

In previous studies, the possibility of using solvatochromic dyes as physicochemical markers of homeopathic medicines was demonstrated,[3–8] allowing the detection of homeopathic activity in aqueous media.[3,4]

Solvatochromic dyes are environmentally sensitive polar dyes that can detect the presence of homeopathic potencies in solvents by variations in their UV-visible spectra.[3–8] This method was developed by one of the authors (S.J.C.) and has resulted in a simple, versatile, and replicable way to use dyes as probes to identify homeopathic medicines in water and provide important data that can assist in elucidating their nature.[5]

The employment of solvatochromic dyes as tracking tools for homeopathic medicines in water sources when used for the treatment of animals or plants (crops or natural ecosystems) emerged in 2018 as a result of a technique previously developed by SIGO Homeopatia of pouring homeopathic products into bodies of water in properties and parks in Mato Grosso do Sul, to facilitate the management of large herds and wild animals requiring veterinary treatment.[9] The same rationale was used at CRAS in the current study. The application of homeopathy in agriculture to control diseases caused by various species of microorganisms and insects has been increasingly studied by researchers and used by farmers.[9–11] Tracking homeopathic products in small[12] or large[13] volumes of water using solvatochromic dyes as markers is a focus of continuing interest. The method was first tested in Brazil by Aparicio et al,[13] whose results showed these dyes to be a high-sensitivity method for detecting homeopathic medicines in lakes.

In the present study, this method was applied to identify and track homeopathic potencies in water where samples were collected in the park facility at different times and locations. Based on evidence that the interaction between dye and potency probably occurs through a field effect rather than by a molecule-to-molecule interaction,[8] water samples were analyzed by two methods: untreated, and subjected to a strong and unidirectional magnetic field before assaying. This approach was based on the hypothesis that magnetic fields may facilitate the field effect between dye and potency.[12] Such an approach is supported by the observation that water subjected to magnetic fields appears to produce specific biological effects in agricultural contexts.[14]

In summary, this real-life case study was an opportunity to verify the possibility of tracking homeopathic signals (FPU) in water samples collected from a multiple park facility at different times and places by using solvatochromic dyes.

Materials and Methods

Information about the Area and Intervention Strategy

CRAS is located in the Prosa Park, as described in Introduction (►**Fig. 1**).

The homeopathic complex FPU was prepared on a solid base placed inside biodegradable devices suitable for

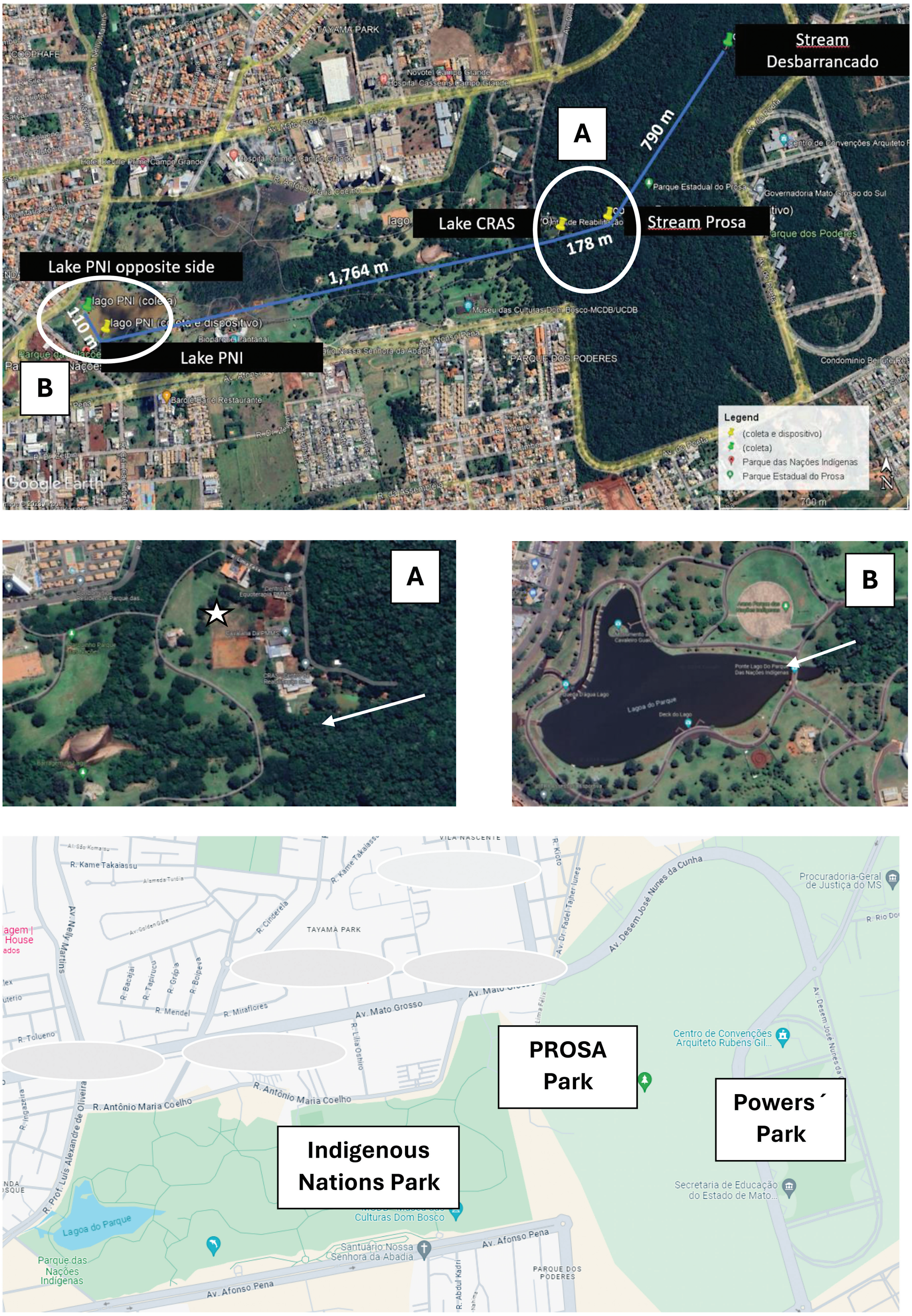

Fig. 1 Satellite image showing where the devices with the homeopathic complex were installed. The blue lines indicate the distance between the placemarks in meters. Yellow marks represent the places where the devices were installed, and water samples were harvested (Lake CRAS, Prosa, and Lake PNI). The green marks represent the places of water sampling only (Stream Desbarrancado and Lake PNI opposite side). The details show the CRAS area (marked with a white star) inside the Prosa park (**A**) and the lake of the Indigenous Nations Park (**B**). The water flow goes from Stream Desbarrancado to Lake PNI under the trees (*dark green areas*), as shown by the white arrows. The map below shows the three parks' general aspect and boundaries. (Adapted from Google Earth Pro and Google Maps, 2023).

Table 1 Schedule of dates and the points where the homeopathic complex devices were installed. The locations of water sampling are also indicated.

Date	Device installation	Sampling before installation	Sampling after installation
September 30, 2021	• Lake CRAS	• Lake CRAS	• Lake CRAS • Lake PNI (right side)
October 10, 2021	• Lake PNI (right side)	________	• Lake CRAS • Lake PNI (right and opposite side)
December 12, 2021	• Stream Prosa • Lake CRAS	• Stream Prosa • Lake CRAS	• Stream Desbarrancado • Stream Prosa • Lake CRAS
January 11, 2022	_________	_________	• Stream Desbarrancado • Stream Prosa • Lake CRAS • Lake PNI (right and opposite side)

fixation. One device was installed in Lake CRAS on September 30th, 2021, and water samples were harvested at two sequential points (Lake CRAS and Lake PNI) before and after its installation. On October 8th, 2021, another water sample was taken at both points, and a new device was installed at Lake PNI. After the natural dissolution of this first set of devices, a second set of FPU complexes was installed on December 12th, 2021, at Stream Prosa and Lake CRAS. Water samples were taken at Stream Desbarrancado, Stream Prosa, Lake CRAS, and Lake PNI on January 11th, 2022. The schedule of treatments and when samples were taken was determined by the park staff according to the animals' needs, as shown in ►**Table 1**.

Ethics and Regulations

There is no legal restriction for homeopathy use in soil or water in Brazil, according to Regulatory Instruction number 52 (March 15th, 2021),[15] as the risk of chemical contamination is zero. The authorization for the treatment of ecosystems is expressed by the Technical Cooperation Term No. 004/2020, signed between the IMASUL and the company SIGO Procedimentos Homeopaticos Ltda. (or SIGO Homeopatia), process No. 71/403033/2020. The company SIGO has an inter-disciplinary team that carries out routine activities related to all philanthropic projects (Amigo SIGO program).

To proceed with the water sample analysis, a second technical cooperation term was signed between SIGO Homeopatia and the Vice-Reitoria de Pós Graduação, Universidade Paulista (UNIP), on November 1st, 2022.

The Homeopathic Complex and Controls

FPU, a homeopathic complex, was prepared to treat tapirs (*Tapirus terrestris*) and capybaras (*Hydrochoerus hydrochaeris*) living in the area. The homeopathic complex was produced by the company SIGO Homeopatia exclusively for this purpose. It followed the standards of good handling practices described in the Brazilian Homeopathic Pharmacopoeia, 3rd edition.[16]

The homeopathic complex FPU was formulated based on the clinical experience of M.F.A.S. (one of the authors). It contains the following medicines: *Chamomilla* 12cH, *Artemisia absinthium* 12cH, *Calcarea carbonica* 30cH, *Psorinum* 12cH, *Sulphur* 12cH, *Ledum palustre* 12cH, *China* 12cH, and *Apis melifica* 12cH. All are prepared separately and then mixed in equal parts, as recommended.[16] The rationale used to create this formulation was:

(1) Psora-focused remedies for the control of ectoparasites, itching and skin lesions caused by insect bites: *Psorinum*, *Sulphur*, *Ledum palustre*, *China*, and *Apis mell.*
(2) Stress control: *Artemisia absintium* and *Chamomilla*.
(3) Improvement of growth and development: *Calcarea carbonica*.

Hard gel tablets made with calcium bicarbonate and hydrocolloid (►**Fig. 2**), measuring 5.1 cm in diameter and 2.5 cm high, with a lifespan of around 60 days, were each soaked with 10 mL of the homeopathic complex. A twin set was placed in a biodegradable device (►**Fig. 3**) with a lifespan

Fig. 2 Hard gel tablets (hydrocolloid and calcium bicarbonate) are used to dispense the homeopathic complex, which is inserted into the devices after soaking. Image courtesy of Mônica F Souza.

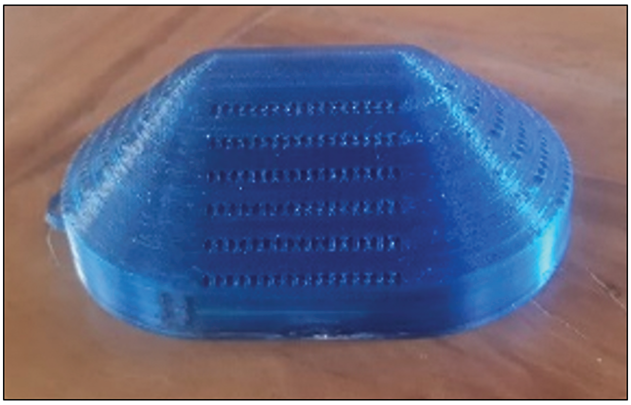

Fig. 3 Biodegradable device used to dispense the homeopathic complex. Lifespan of 1 year. Image courtesy of Mônica F Souza.

of 1 year. Both components (gel and biodegradable cage) were created and patented by SIGO Homeopatia owners, and their exact composition is under copyright protection. They were designed to be residue-free, using only organic components such as hydrogel and calcium carbonate; this last one is a component often used as a solid base for homeopathic commercial preparations for veterinary use. Thus, there is no possibility that these components interfere with the results, considering that solvatochromic dyes are sensitive to the excited state of succussed polar solvents only.[5,6]

At the time of analysis, samples of FPU kept in stock in liquid form were filtered with a 0.22 µm mesh filter (Merck–Millipore, Darmstadt, Germany) to rule out any microorganism or debris that could interfere with the spectrophotometer reading. Then, they were diluted in a 1:100 ratio in 30% ethanol (HN Cristiano, São Paulo, SP), filtered (as previously explained), and stored in newly acquired autoclaved amber vials. This procedure was performed in a laminar flow to keep the sample handling environment sterile. Then, these vials were labeled, and 100 succussions were done in a mechanical arm (Denise, AUTIC, São Paulo, Brazil) able to perform a semi-circular movement, with a 55° angle, 300 mm radius, with operational cycle equivalent to 100 pulses in 33 seconds, 1,620 rpm/880 g, according to manufacturer information. These standards generate a calculated force of approximately 2,420 N. After this procedure, the vials were packed with aluminium foil, isolated from any electromagnetic field, and kept at stable room temperature.

The 30% non-succussed ethanol (vehicle) was used as a negative control for the laboratory *in vitro* step of the study. *Ethilicum* 1cH (succussed ethanol) was used as a parallel comparative control.

Collection and Handling of Samples

In addition to the samples of the FPU homeopathic complex, water samples taken from different locations on different days (before and after the placement of the devices containing the homeopathic complex) were also evaluated. The choice of days and times for placing the devices and for water sampling was arbitrarily defined according to the routine observations of the park technicians, with the assistance of SIGO Homeopatia veterinarians. The rationale used to choose the places for the device insertion was based on the natural water flow in the area, from the Desbarrancado source to the PNI point, as shown in ►**Table 1** and ►**Fig. 1**.

Water samples were stored in sterile plastic containers wrapped in aluminium foil and frozen at −10°C. Before sending the samples for analysis, they were thawed, filtered in a 0.22 µm mesh filter (Millipore, Burlington, Massachusetts, United States), and packed in 1.5 mL microtubes, being re-frozen until the moment of analysis. All samples were coded and labeled before being sent to the laboratory for analysis.

When thawed, they were diluted in a 1:100 ratio in 30% ethanol (HN Cristiano, São Paulo, SP), also filtered in a 0.22 µm mesh filter (Merck–Millipore, Darmstadt, Germany), and stored in newly acquired autoclaved amber vials (30 mL). This approach respects the standard protocol for water analysis established previously,[4,13,17,22] whose aim is to ensure an excited stage of the polar solvent by dilution/succussion since it is an essential feature for the interaction with these dyes.[5,6] This procedure was also performed in laminar flow in a sterile environment. The toggles and other non-autoclavable materials were cleaned with 70% alcohol and left in the laminar flow under UV light for 15 minutes before being used.

These vials were then labeled and succussed 100 times in a mechanical arm (Denise, AUTIC, São Paulo, Brazil), as described above. After this procedure, the vials were labeled with the same codes assigned previously, packed with aluminium foil, insulated from any electromagnetic field, and kept at room temperature.

Spectrophotometry and Solvatochromic Dyes

Samples of liquid stock FPU were analyzed at the Extraction Laboratory—UNIP, São Paulo. The facilities used were a laminar flow cabinet, a spectrophotometer with millesimal sensitivity, and a scan of at least 1 nanometer (FEMTO 800 XI, São Paulo, Brazil). A precision scale was used for weighing of powder dyes (METTLER—Toledo Monobloc Classic Line AB240-S, Greifensee, Switzerland).

Six solvatochromic dyes were tested: coumarin 7, rhodanine, Nile red, BDN (4-[bis-[4-[dimethylamine] phenyl] methylene]-1[4H]-naphthalenone), methylene violet, and N,N-dimethylindoaniline (NN-DMIA), prepared to comply with the methods and concentrations described by Cartwright[5–7] and previously standardized in our laboratory. The samples were tested in triplicate, in three series, with $N = 9$ for each sample. After weighing the dye powder, it was diluted in PA ethanol (Synth, Diadema, Brazil) according to the standard concentrations shown in ►**Supplementary File S1** (available online only), and labeled and stored in a refrigerator at 4°C in Falcon flasks wrapped in aluminium foil to protect the dye from light. For each round of analyses, a new batch of dye was prepared.

On the days of the experiments, the dyes, FPU sample, and 30% alcohol (*Ethilicum*) were previously stirred vertically 30 times, mimicking the Denise robotic arm (AUTIC, São Paulo, Brazil) movements immediately before being aliquoted and analyzed, also generating a calculated force of approximately 2,420 N.

The samples were then filtered with a 0.22 µm mesh filter (Millipore, Burlington, Massachusetts, United States) to rule out any particulate element. The dyes were kept in Falcon flasks labeled and wrapped in aluminium foil, and the filtered samples were placed in microtubes. Then, dyes were placed in disposable cuvettes for reading, in the amount of 1,475 µL for 25 µL of the sample (ratio 1:60). All these materials have been kept covered with aluminium foil to prevent the alteration of the dyes, which are sensitive to light.

The reading was performed immediately after the preparation of all cuvettes using a spectrophotometer (FEMTO 800 XI, São Paulo, Brazil). A bucket with PA ethanol (Synth, Diadema, Brazil) was initially read to calibrate the equipment for each dye. Then, each pure dye was read to determine the absorbance peak and the respective wavelength. This reading was made by scanning the visible light spectrum, whose wavelengths vary between 350 and 800 nm.

Coumarin 7 was selected following the above procedure (►**Supplementary File S2**, available online only) since it produced the clearest results with FPU, as shown in the results. Due to the high number of samples, water readings were performed in microplates (COSTAR, Davis, California, United States), avoiding alcohol evaporation during the experimental procedures since all samples and replicates can be read at once. Thus, 4 µL of sample and 236 µL of coumarin 7 were added to each well and read in an enzyme-linked immunosorbent assay (ELISA) spectrophotometer (Epoch-BioTek, Agilent, Santa Clara, California, United States).

In all cases, the samples underwent 30 additional manual succussions immediately before being aliquoted and analyzed, again mimicking the Denise device mechanical arm movements. They were then filtered with a 0.22 µm mesh filter and placed in 2.0 mL microtubes until their use.

Based on Mohammad et al's study,[14,17] samples (homeopathic complex, water samples, and controls) were treated with an average static and unidirectional magnetic field of 2,400 Gauss. The sample microtubes were placed inside a beaker positioned on the positive surface of a neodymium magnet (NdFeB) model N52, 240 mT, measuring $5.08 \times 5.08 \times 1.27$ cm (MAGNETUM *Produtos Magnéticos*, São Paulo, Brazil), for 15 minutes. Then the samples were pipetted into the microplates, and the reading was done in the ELISA spectrophotometer (Epoch-BioTek, Agilent, Santa Clara, California, United States) immediately after each microplate preparation (►**Supplementary File S3**, available online only). The samples were analyzed in quintuplicate, in two experimental replications, resulting in $N = 10$ for each sample. All samples were run at the same time, independently of the harvesting date. The experiments were run in parallel on the same day.

During all experimental procedures, the temperature, humidity and magnetic flux inside the laminar flow cabin were registered (temperature 25.5 ± 1.31°C, humidity 34.5 ± 12%, and magnetic field 0.13 ± 0.06 µT). A thermo-hygrometer (Tomate PD-003, São Paulo, Brazil) and a Gaussmeter (frequency range: 30–300 Hz, resolution 0.01–0.1 µT, 3% precision at 50–60 Hz—Instrutherm DRE 050, São Paulo, Brazil) were used.

Statistical Analysis

Delta absorbance (the absorbance of each sample plus dye minus the absorbance of the dye alone) was used as data points in the statistical analysis to evaluate the sample–dye interaction.

Windows Prism 10.0 software was used for the statistical analysis. The Shapiro–Wilk test was used to check data Normality, and deviations were normalized by Q–Q plot inspection. One-way analysis of variance followed by the Tukey method was performed to evaluate differences among groups concerning the absorbance delta. The Tukey box-plot mode in Prism 10.0 software automatically identified outliers, which were removed.

Results

Selection of Dyes

Among the six dyes tested, coumarin 7 proved to be the most suitable marker for the FPU homeopathic complex. The dye presented a statistically significant difference between FPU and the negative control, 30% non-succussed alcohol. *Ethilicum* 1cH was less specific in its interactivity with the dyes (►**Fig. 4**).

The same samples were tested again using the magnet/microplates/ELISA reader protocol as a method control for water samples analysis with coumarin 7 as a chosen dye. The same result was observed but with less variance and larger delta values (►**Fig. 5**).

Analysis of Water Samples before and after the Treatment with the Homeopathic Complex FPU

In the September 2021 series, water samples from Lake CRAS showed early changes in coumarin 7 absorbance 1 minute after insertion of the device into the watercourse. This difference was even more evident after 8 days, strongly suggesting the persistence of the FPU signal while the device was immersed. Equivalent delta results were seen in the second round, made in December 2021, though the absorbance decreased in relation to the pre-treatment samples in this case (►**Fig. 6**). Such temporary reversals in the *in vitro* effects of homeopathic medicines have been observed previously[3,18] and have yet to be explained.

The trajectory of the homeopathic signal was checked following the water flow through Lake CRAS to Lake PNI on different days (►**Fig. 7**). There was an increase in the coumarin 7 absorbance in samples obtained from Lake PNI after 75 minutes and 8 days from the device installation at Lake CRAS, indicating traceability as a function of time and distance. On day 8, a second device was installed at Lake PNI, but the response in the following days presented a high variance, reducing statistical significance. A third device was installed at Lake CRAS after 81 days from the first insertion, and the absorbance delta of coumarin 7 in the presence of water samples from PNI remained constant even at day 102.

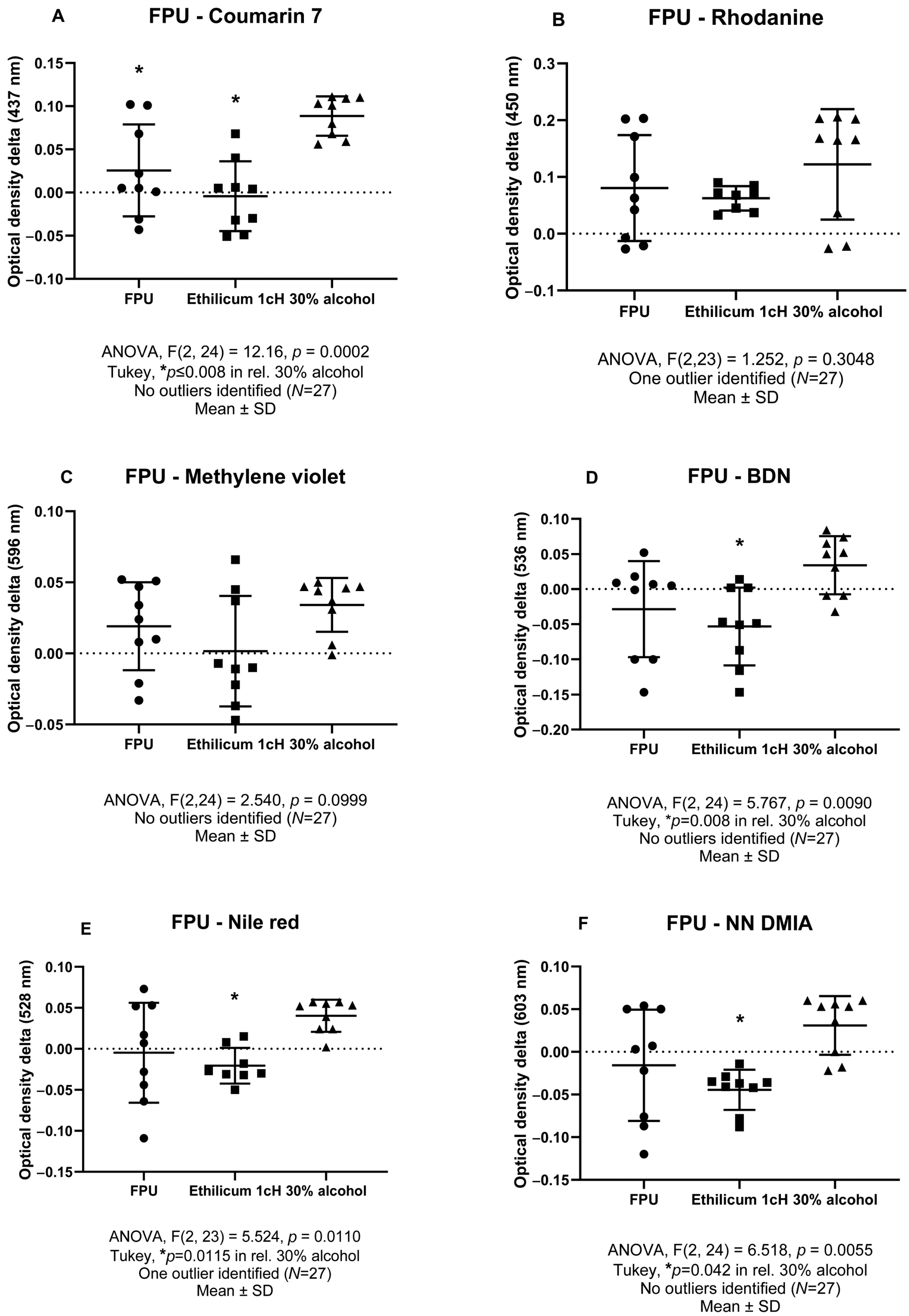

Fig. 4 Screening of solvatochromic dyes (see ►**Supplementary File S1** and ►**Supplementary File S2** for details) for the selection of a marker for the homeopathic complex FPU (*Formula Parques Urbanos*). The statistical data are presented in each graph as mean ± standard deviation and individual values. $N = 9$ for each group.

In the Desbarrancado and Prosa stream cases, no samples were available on the first day prior to the device installation. Because of this, samples harvested on December 12th, 2021 and January 11th, 2022 (day 32) were analyzed as single points, with no pre-treatment corresponding pairs (►**Fig. 8**). There was an early and transitory statistically significant difference in the absorbances in both cases.

Discussion

In line with the concept of One Health,[19] environmentally friendly and non-toxic treatments to control insect infestations need to be developed. Based on three previous real-life studies in the wild,[13,20,21] homeopathy could be considered an environmentally sustainable approach to balance

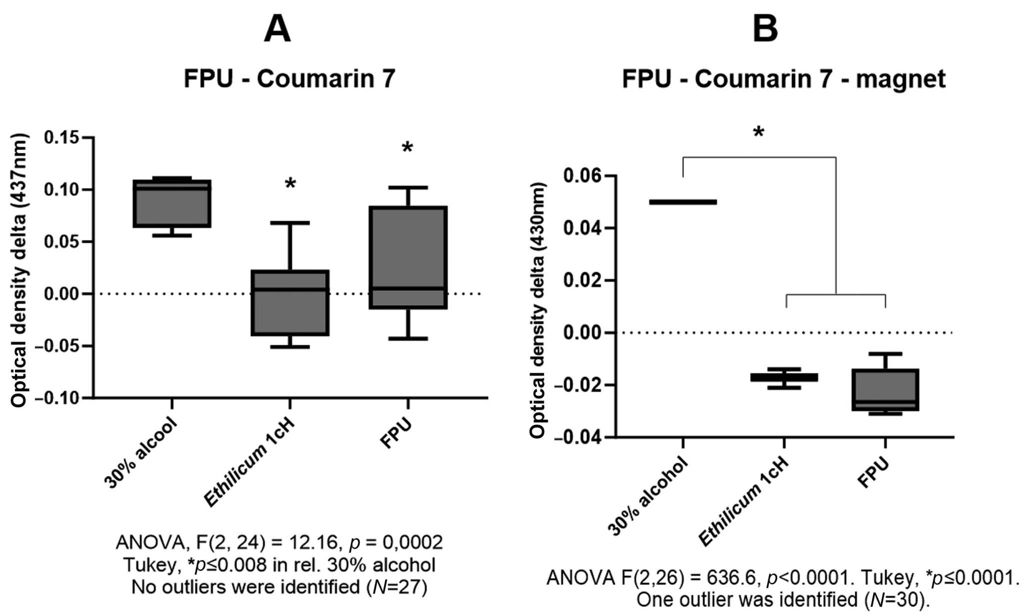

Fig. 5 Boxplot of coumarin 7 absorbance delta in the presence of homeopathic complex (FPU) and the controls, *Ethilicum* 1cH and 30% alcohol. (**A**) Results obtained from the microplates/ELISA reader protocol. (**B**) Results obtained from the magnet/microplates/ELISA reader protocol. Statistical data are presented at the bottom of the graph. $N = 9/10$ in each group.

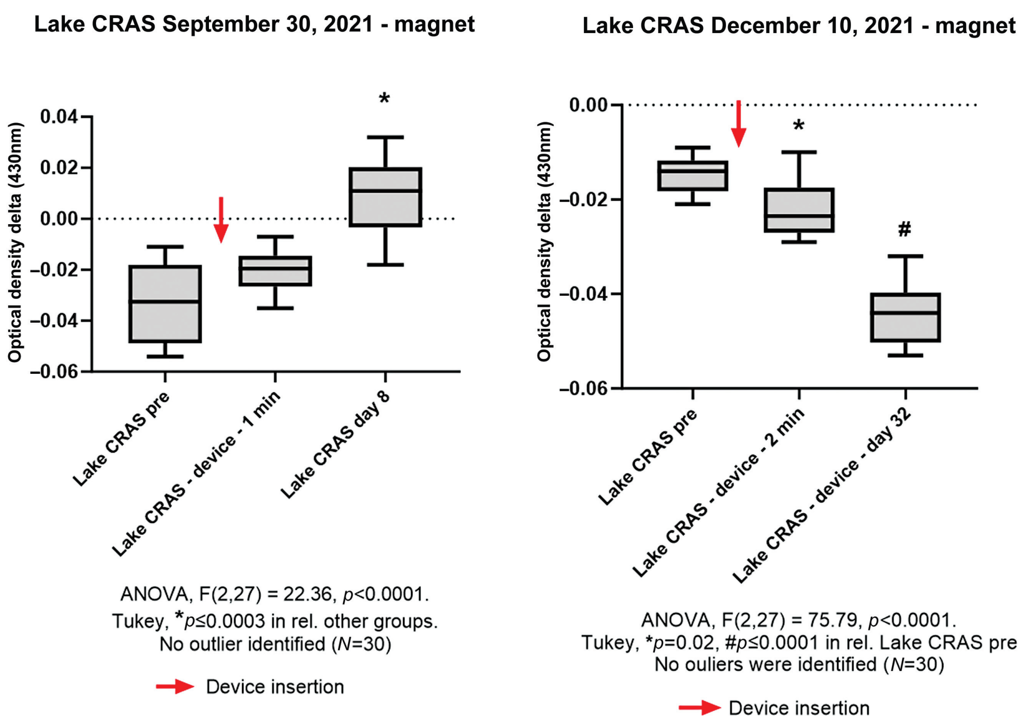

Fig. 6 Boxplot of coumarin 7 absorbance delta, comparing the water samples before and after the device insertion (*red arrows*) at Lake CRAS on September 30th and December 10th, 2021. Statistical data are presented at the bottom of the graph. $N = 10$ in each group.

ecosystems. In these cases, there was the opportunity to collect water samples over time and use solvatochromic dyes to track homeopathic signaling in water, even across large areas. This is the first step in a new line of research, so further studies in this area need to be performed both in laboratories and under field conditions, including those related to agriculture and food production.[9–11]

The present study aimed to evaluate the possibility of tracking a homeopathic complex inserted at different locations within a large park that was having problems with tick infestation. Previous results using solvatochromic dyes to track homeopathic medicines in the wild[3,5,13,20,21] indicate that signal propagation through water occurs very rapidly, and the same observation has been made in the current

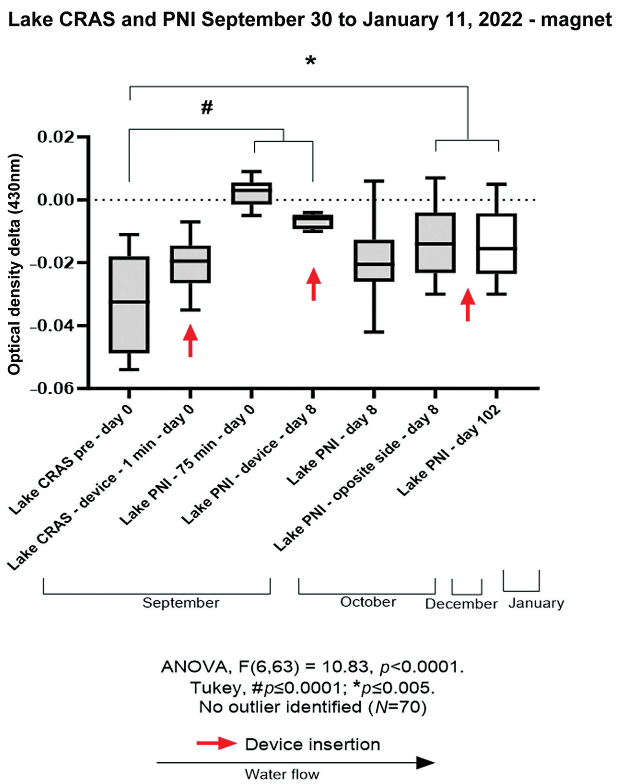

Fig. 7 Boxplot of coumarin 7 absorbance delta, showing the trajectory of the homeopathic signal through Lake CRAS to Lake PNI (*both sides*) between September 30th 2021 and January 11th 2022. Statistical data are presented at the bottom of the graph. $N = 10$ in each group.

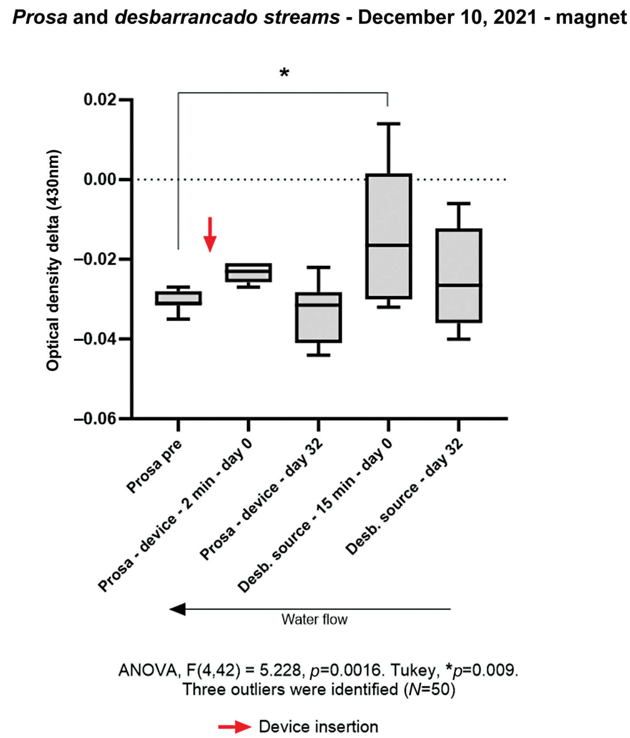

Fig. 8 Boxplot of coumarin 7 absorbance delta, comparing water samples from stream Prosa and stream Desbarrancado (*source*), where water samples were harvested on December 10th 2021 and January 11th 2022. The device was inserted on December 10th 2021 (*red arrow*). Statistical data are presented at the bottom of the graph. $N = 10$ in each group.

work. These are intriguing results that require an explanation. Quantum electrodynamics (QED) may explain how such a small stimulus could propagate throughout large bodies of water via a single rhythmic oscillation over long distances and as a single macroscopic observable phase. According to QED theory, it is postulated that energy can travel in a coherent medium in the form of *solitons* without its loss.[22–25]

The propagation of homeopathic signals in water associated with a field concept is not a new hypothesis. Endler et al[26–29] used closed ampules containing potentized thyroxin, which were introduced into water containing tadpoles. Their enhanced metamorphosis into frogs without direct molecular contact with the homeopathic stimulus has been taken to indicate the possibility of some kind of field effect, possibly magnetic, being involved in the mechanism.

Another question that has emerged during the present study concerning tracking homeopathic medicines by solvatochromic dyes is how a homeopathic complex, composed of several medicines, could act as if it were a remedy prepared from a single substance. Kokornaczyk et al[30] have argued that homeopathic complexes behave as unique medicines from the physico-chemical point of view. There seems no reason, therefore, why they could not be tracked by the dyes as if they were a single-substance-made medicine. Present and past results support this conclusion.[3,4,12,13,17,22]

From the six dyes tested, coumarin 7 was found to be the most suitable marker for the homeopathic complex formulated to treat the park in the present study. Consequently, this dye was used to analyze samples taken from the park's water system. To improve the method, the technique of applying a magnetic field to samples prior to assay and reading multiple results using microplates simultaneously with an ELISA spectrophotometer was used. Since the microplates allow reading several samples at once, any bias due to alcohol evaporation (coumarin 7 is prepared in PA ethanol) was avoided. In fact, this protocol proved to be more effective in making clear the differences in absorbance between verum and controls, as already reported[17] and shown herein. Method sensitivity could also be increased after samples were submitted to a magnetic field. We postulate that magnetic fields may facilitate the interaction between solvatochromic dyes and potencies, and this postulate is supported by the observations of Nandy et al.[31]

The specificity of coumarin 7 is another interesting point that needs to be discussed. We have seen, in previous results, that there is a relative specificity between dyes and potencies in such a way that, in each assay, it is possible to find the best dye to be used as a marker regarding the remedy and the potency.[3,12,17] For example, coumarin 7 is also an excellent marker for *Glyphosate* 6cH in water with different degrees of salinity.[22] Methylene violet has been identified as a marker for many kinds of homeopathic preparation, such as complexes or single medicines prepared from *Phosphorus*, *Silicea terra* or *Antimonium crudum*.[3,4,13] Due to this feature, a prior dye screening was established as a standard method every time a new homeopathic potency is tested.

Despite the natural limitations and difficulties of obtaining results in a real-life situation, we have shown it is possible to track homeopathic signals in water, even in high volumes and over large distances, as shown in ►**Fig. 7**.

In addition, the new technology, which uses a calcareous base and a biodegradable device to contain the homeopathic medicines, extended the time during which the medicines were active and detectable, compared with previous results.[13] At the Lake CRAS installation point, tracking was possible even after 8 days following the insertion of FPU, and this result was present in both the September and December 2021 FPU installations, as shown in ►**Fig. 6**. Replacing some of the devices over time also extended the tracking period (►**Fig. 7**). However, where the devices were not replaced, the signal faded after 32 days (►**Fig. 6**). In a previous study,[13] *Phosphorus* 30cH was tracked following a single liquid treatment at the source of a lake system but was found to fade after 48 hours.

The most intriguing result is shown in ►**Fig. 8**. Even though it has a transitory effect, FPU was tracked in water in the opposite direction to water flow. This observation suggests that homeopathic signal propagation does not depend on water flow and supports the hypothesis of some kind of field effect. However, further laboratory and field studies comparing tracking before and after device immersion into the water at different points (with and against water flow) need to be performed to exclude completely the possibility of this finding being an accidental one due to uncontrolled sample interference. The present result may inspire other authors to develop this line of research in different countries and under different conditions, which would be crucial for developing agricultural and environmental management standards using homeopathy and high-dilution technology.

Besides the results presented here on tracking the FPU homeopathic complex in water resources, the clinical outcome of the tick infestation control was presented and recorded in the Proceedings of the XXXVI GIRI meeting 2023,[21] showing that the tick infestation control appeared successful in this case. However, more field studies using a trial design that includes a negative control are still needed to validate this kind of technology in treating wild animal populations.

Nevertheless, the results described herein suggest that those homeopathic devices allied with the solvatochromic dyes method could be an environmentally sustainable strategy to control outbreaks in wild conditions, with the advantage of tracking the homeopathic signals in water for controlling posology, making this process more efficient.

Conclusion

Coumarin was found to be the most suitable marker for the FPU homeopathic preparation used to treat this multiple park facility and its animals. Analyzing water samples taken at different times and geographic points showed the plausibility of tracking homeopathic signaling in field conditions using solvatochromic dyes, even over large distances. Using a solid device previously embedded with the liquid homeopathic formulation and immersed in water resulted in persistence of the signal for some days. Replacing the devices extended this time. In addition, signal propagation seems not to depend on the direction of water flow, though this issue needs further systematic studies to be confirmed, clarified and extended.

Highlights

- Solvatochromic dyes can track homeopathic complexes in large volumes of water.
- Following the screening of several dyes, coumarin 7 was found to be the most suitable for tracking the FPU homeopathic complex in the natural park setting of this study.
- The use of solid devices embedded with the homeopathic preparations leads to stable signal propagation in water.

Supplementary Material

Supplementary File 1. The solvatochromic dyes.
Supplementary File 2. Coumarin 7 visible light spectrum.
Supplementary File 3. Experimental design.

Authors' Contributions

N.S.S.M.—Main researcher, postgraduate student, involved in most of the experimental procedures.
A.A.G.P.—Experimental procedures—solvatochromic dyes tests.
S.F.—Experimental procedures—solvatochromic dyes tests.
M.F.S.—FPU formulation; treatment strategy; water sampling.
I.B.S.—Experimental procedures—solvatochromic dyes tests.
S.J.C.—Experimental design with solvatochromic dyes.
L.V.B.—Main Adviser, coordination of all steps of the study, and discussion of results.

Conflict of Interest

The samples were made available for analysis by SIGO Homeopatia company in partnership with the governmental organization IMASUL (Instituto do Meio Ambiente do Mato Grosso do Sul). No other resource was given to the researchers.

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