Tracking a Homeopathic Complex Formulation in the Watercourses of a Fire-Damaged State Park in Brazil

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Abstract

Introduction In 2020, a 26,849-ha state park in Mato Grosso do Sul state, Brazil, had 30% of its area damaged by fire. A homeopathic complex formulation was applied at strategic point locations in the park's springs or watercourses, aiming to mitigate the fire damage to the flora and fauna as quickly as possible. The duration of the homeopathic signal at each point was assessed using an established solvatochromic dye technique.

Objective To evaluate the timing and the nature of the signal at each of nine point locations. We could thus identify the presence of any signal variations due to specified environmental features within the park.

Methods Water samples were harvested from each intervention point at different times, filtered, frozen, and sent to the laboratory, where they were prepared to 1cH using filtered 30% ethanol. Methylene violet was chosen among six dyes since it was found in preliminary tests that it could trace the homeopathic complex used. In addition to simple sample testing, samples were submitted to a static and unidirectional magnetic field of 2400 Gauss (240 mT) for 15 minutes immediately before reading, which enhanced the method's sensitivity. One-way analysis of variance/Tukey test was used to identify dye absorbance changes following the analysis of water samples from the watercourse system. A correlation matrix and the Spearman *r* test were employed to evaluate any correlation between tracking and the pre-existing anthropic interventions at harvesting points. In all cases, $\alpha = 0.05$.

Results Four tracking patterns using the sample magnetization process were observed in relation to water samples and their effect on methylene violet solutions: no response (P2, P4), early transitory response (P5, P6, P8), late response (P1, P9), and

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- watercourses
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- anthropic impact

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constant response (P3, P7). P2 and P4, which could not be tracked, were correlated with permanent local anthropic disturbance.
Conclusions Methylene violet was the best dye to track the homeopathic complex prepared specifically for this case. Tracking was facilitated by prior magnetic treatment of samples, but anthropic disturbances to the environment seem to interfere with it.

Introduction

The use of homeopathic preparations in watercourses to improve food production in agriculture and husbandry and promote wild animal health has been recently developed in Brazil. In this context, our group in particular has focused on refining the treatment strategy by tracking homeopathy activity in water by using the solvatochromic dyes method.[1–3]

In 2020, a new demand emerged. During the dry season, a fire in the *Parque Estadual das Nascentes do Rio Taquari*—PENT (in English, Taquari River Springs State Park) in Mato Grosso do Sul state, Brazil, destroyed almost 30% of its total extent. Fires are frequent during this area's dry seasons and are common in the Brazilian savannah biome.[4] Following a technical cooperation agreement (TCA) between the Institute for the Environment of Mato Grosso do Sul (IMASUL) and the company *Sigo Homeopatia*, a formulated homeopathic complex (*Formula Parque Taquari*—FPT) was designed and prepared specifically for this case. The rationale for treatment centered on homeopathy-related keywords that connected biological fire damage with corresponding remedies, as described in homeopathic materia medica and repertory (details in Materials and Methods section). The intervention had the aim of promoting faster post-fire recovery of the biome's variously affected flora and fauna.[3,5]

The approach was inspired by laboratory studies on water contamination showing toxicity mitigation by isotherapeutics[6,7] and microorganism growth control by specific homeopathic preparations.[8,9] In all cases, tracking homeopathic preparations in water was possible by means of solvatochromic dyes, in which high specificity and sensitivity levels were observed.[6–9]

Homeopathic preparations are produced by serial dilutions of active substances in polar solvents followed by vigorous and repeated vertical agitation, a process known as succussion. This process creates a great diversity of physicochemical consequences, such as the generation of oxygen-free radicals associated with nanobubbles,[10] nano- and microparticle consolidation,[11–13] and changes in solvent dipole moment,[14–17] which, it is postulated, may be related to the electrodynamic structuring of a polar solvent such as water, as proposed by Del Giudice's team[18–23] and other authors.[24,25]

This background allowed one of us (S.J.C.) to create, in 2016, a simple, versatile, and replicable method in which solvatochromic dyes are used as probes to identify homeopathic medicines in polar solvents, as well as providing important data that have assisted in elucidating their nature.[14] Solvatochromic dyes are electronic dyes that can detect changes in solvent polarity and electric field strengths, among other environmental parameters.[14–17,26,27] In view of the hypothesis that the process of serial dilution and succussion of a given substance may modify the physicochemical properties of solvents, and in particular water, solvatochromic dyes have been proposed as sensitive probes capable of identifying succussion-induced solvent changes. Subsequent studies have demonstrated the effectiveness of solvatochromic dyes in detecting and tracking homeopathic medicines, specifically under laboratory conditions,[26–28] and with a high degree of specificity.[29]

The proposal to adopt the solvatochromic dye method as a tracking tool to follow homeopathic preparations in water sources—natural or artificial—during the treatment of mammalian herds, aquatic animals, or crops,[30–34] emerged in 2018[2] based on the method previously developed by Souza to facilitate the management of large herds treated with a homeopathic remedy that was added to their drinking water.[30] In the current study at PENT, water samples from the nine intervention points used in this case were harvested to proceed with the tracking analysis. Our objective was to determine the timing and the nature of the signal at each point: we were thereby able to gain an understanding of any signal variations due to specified environmental features of the state park.

Materials and Methods

Study Design

This study aimed to identify the presence of the homeopathic complex FPT in water at different times, before and after the insertion of the intervention device (FTP-embedded solid base) at different point locations in the watercourses. To achieve this objective, water samples were harvested to be evaluated by solvatochromic dyes, following a similar methodological approach as previously described.[1,9] The choice of the best responsive dye to be used was based on the capacity of each dye to interact with the FPT complex as identified by changes in the dye's absorbance.

Once methylene violet was chosen as the ideal dye (see below), water samples were tested under two different protocols—with or without sample magnetization—to achieve the best sensitivity level.[1,9]

After water analyses, different patterns of response to intervention were revealed. To propose a solid hypothesis to explain this finding, we undertook a statistical correlation between the presence or absence of water–dye interaction and relevant features of the surrounding environment at each intervention point.

Area Characterization

This 26,849-ha park is located on the Guarani and Bauru aquifers. It contains hundreds of springs that feed the Paraguay River and is part of the Planning and Management Unit 2.II—Taquari, which unites the Brazilian savannah (called Cerrado) and Pantanal biomes (►**Supplementary Fig. S1**, available in online version only). It is the most important savannah reserve of Mato Grosso do Sul state.[4] The park is under the management of IMASUL.

Ethics and Regulations

For the environmental use of homeopathy in Brazil, there is no legal restriction for its use in soil or water, according to the Regulatory Instruction number 52 (March 15, 2021),[35] given that the risk of chemical contamination in these cases is zero. The authorization for the treatment of ecosystems is expressed by the Technical Cooperation Term No. 004/2020, signed between IMASUL and the company Sigo Procedimentos Homeo–paticos Ltda. (*Sigo Homeopatia*™), process No. 71/403033/2020, as part of the activities concerning its philanthropic project, *Amigo Sigo*. 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 1, 2022.

Production of the Homeopathic Complex

FPT is a homeopathic complex that was designed specifically for the PENT in 2020. The company *Sigo Homeopatia*™ produced it and followed the standards of good handling practices described in the Brazilian Homeopathic Pharmacopoeia, 3rd edition.[36] FPT contains the following components: *Arnica montana*, *Arsenicum album*, *Ignatia amara*, *Phosphorus*, and *Staphysagria*, each at 30cH potency and mixed in equal parts.

The rationale for the formula was based on keywords representative of each remedy as described in homeopathic materia medica and homeopathic repertory. The corresponding keywords were based on the expected effects of fire on plants and/or animals. References are shown in ►**Table 1**.[37–41]

The production of FPT was based on the use of a solid base (dry *Lithothamnium calcareum*—a porous calcium solid base) soaked by pouring 10 mL of the homeopathic complex (►**Fig. 1A, B**). These devices were fixed to riparian plants to keep themselves immersed in water at each intervention point. They were inserted into water over the course of 10 days, between September 29 and October 11, 2020. The choice of days and times for placing the devices and collecting water sample frequency was defined according to the routines of the park technicians (IMASUL). They performed the insertion and monitoring of the devices and water harvesting under the coordination of author A.P.F.

Water samples were harvested just before and after (immediately, 24, 48, and 72 hours) the immersion of the device at each point to track the signal as a function of time, point by point. In some cases, one or two sampling times were skipped due to accessibility limitations. The harvesting procedure followed the standards described by Aparicio et al, 2020.[2] These devices were collected to be recycled after the last water sample was harvested, avoiding water pollution.

Collection and Handling of Samples

Water samples were taken before and after the devices were inserted into the nine points at different times to follow the homeopathic signal. IMASUL and *Sigo Homeopatia*™ kindly provided the water samples for the analyses to be done at the Research Center, University Paulista (UNIP), São Paulo, after another TCA was signed between UNIP and *Sigo Homeopatia*™ to proceed with the present study.

At the time of analysis, samples of the homeopathic complex from the same batch (kept in stock in liquid form) and pure water (control) were filtered with a 0.22-µm mesh filter (Merck–Millipore, Darmstadt, Germany) to rule out any bacteria or micro-debris that could interfere in the light beam during the spectrophotometer reading.[1,2,9] Then, they were diluted in a 1:100 ratio in 0.22-µm mesh filtered 30% ethanol (HN Cristiano, São Paulo, Brazil) and stored in newly acquired autoclaved amber vials (final volume: 20 mL). This procedure was performed in a laminar flow to keep the

Table 1. Components of the homeopathic complex *Formula Parque Taquari*, keywords described in materia medica and repertory, and corresponding references

Homeopathic component (each at 30cH)	Keywords	References
Arnica montana	Burns/trauma	Ribeiro Filho[37]
	Traumatic events	Guermonprez et al[38]
Arsenicum album	Burns	Ribeiro Filho[37]; Vijnovsky[39]
	Necrosis and ulcerations	Guermonprez et al.[38]
Ignatia amara	Burns	Ribeiro Filho[37]
	Traumatic events	Guermonprez et al.[38]
Phosphorus	Burns/trauma/tissue disruption/necrosis	Guermonprez et al.[38]; Ribeiro Filho[37]
Staphysagria	Tissue disruption	Boericke[40]
	Trauma	Ribeiro Filho[37]
	Vital fluid loss	Scholten[41]

A B

Fig. 1 (**A**) Dry *Lithothamnium calcareum*. (**B**) A grid container filled with *Lithothamnium calcareum* embedded into the FPT complex was used to allow homeopathic signaling in water by fixing it in the riparian plants so that the solid base would be immersed at each intervention point. FPT, *Formula Parque Taquari*.

sample-handling environment sterile. Clean first-use toggles and other non-autoclavable materials were left in the laminar flow under ultraviolet (UV) light for 15 minutes before use. Then, these vials were labeled and submitted to 100 successions in a mechanical arm (Denise, AUTIC, São Paulo, Brazil) able to perform a semi-circular movement, with a 55-degree angle, 300 mm radius, with operational cycle equivalent to 100 pulses in 33 seconds, 1620 rpm/880 g, according to manufacturer information. These standards generate a calculated force of approximately 2420 N. After this procedure, the vials were packed with aluminium foil, isolated from any electromagnetic fields in a closed cabinet, and kept at stable room temperature.

As a negative control, filtered 30% non-successed ethanol was used. Filtered 30% successed ethanol (or *Ethilicum* 1cH) was used as a comparative control. The preparation method was the same as described above.

In addition, water samples harvested in PENT from different points, days, and times (before and after placing the devices with the homeopathic complex, as described above) were similarly processed. These samples were stored in sterile plastic standard containers, wrapped in aluminium foil, and frozen at −10°C. Before the analyses, they were thawed, filtered in a 0.22-µm mesh filter (Merck–Millipore, Darmstadt, Germany), packed in 1.5 mL microtubes, coded, and re-frozen until the moment of analysis.[1,2] When thawed, they were diluted in a 1:100 ratio in filtered 30% ethanol (HN Cristiano, São Paulo, Brazil) and stored in newly acquired autoclaved amber vials (final volume: 20 mL). This procedure was also performed in a laminar flow in sterile conditions, labeled, and successed 100 times in a mechanical arm (Denise, AUTIC, São Paulo, Brazil), as described above. Thus, the 1cH of all samples was used in the readings in a standardized manner. Next, these vials were also packed with aluminium foil, insulated from any electromagnetic field, and kept at stable room temperature, following the standards described in previous studies.[1,9] Since the IMASUL–*Sigo* team created the codes in Campo Grande City, a technician who did not participate in the analyses at the UNIP Research Centre in São Paulo City saved the sample descriptions for each code. Thus, all analyses were performed blinded. The flowchart describing the sample handling steps is shown in ►**Fig. 2**.

Spectrophotometry and Solvatochromic Dyes

Choosing the Best Dye to Identify the *Formula Parque Taquari* Complex

Samples of FPT complex were tested in a screening with six solvatochromic dyes: Coumarin 7, Dimethylaminobenzylidene-rhodanine (or 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[14–16] and previously standardized in our laboratory.[1,2,9] The samples were tested in triplicate, in three series, with $N = 9$ for each sample.

After weighing each dye on a precision scale (METTLER—Toledo Monobloc Classic Line AB240-S, Greifensee, Switzerland), it was diluted in PA ethanol (Synth, Diadema, Brazil), filtered in a 0.22-µm mesh filter (Merck–Millipore, Darmstadt, Germany), and stored in Falcon flasks, which were wrapped in aluminium foil, labeled, and stored in a refrigerator.

On the days of the experiments, FPT and control samples were manually stirred vertically 30 times, mimicking the Denise device's (AUTIC, São Paulo, Brazil) mechanical arm movements. This generated an approximate calculated force of 2420 N. After this, they were filtered through a 0.22-µm mesh filter to rule out any microparticulate element.

The dyes were also filtered through a 0.22-µm mesh filter before being transferred to disposable cuvettes, in the amount of 1,475 µL of dye for 25 µL of each sample (ratio 1:60). All this material was kept covered with aluminium foil

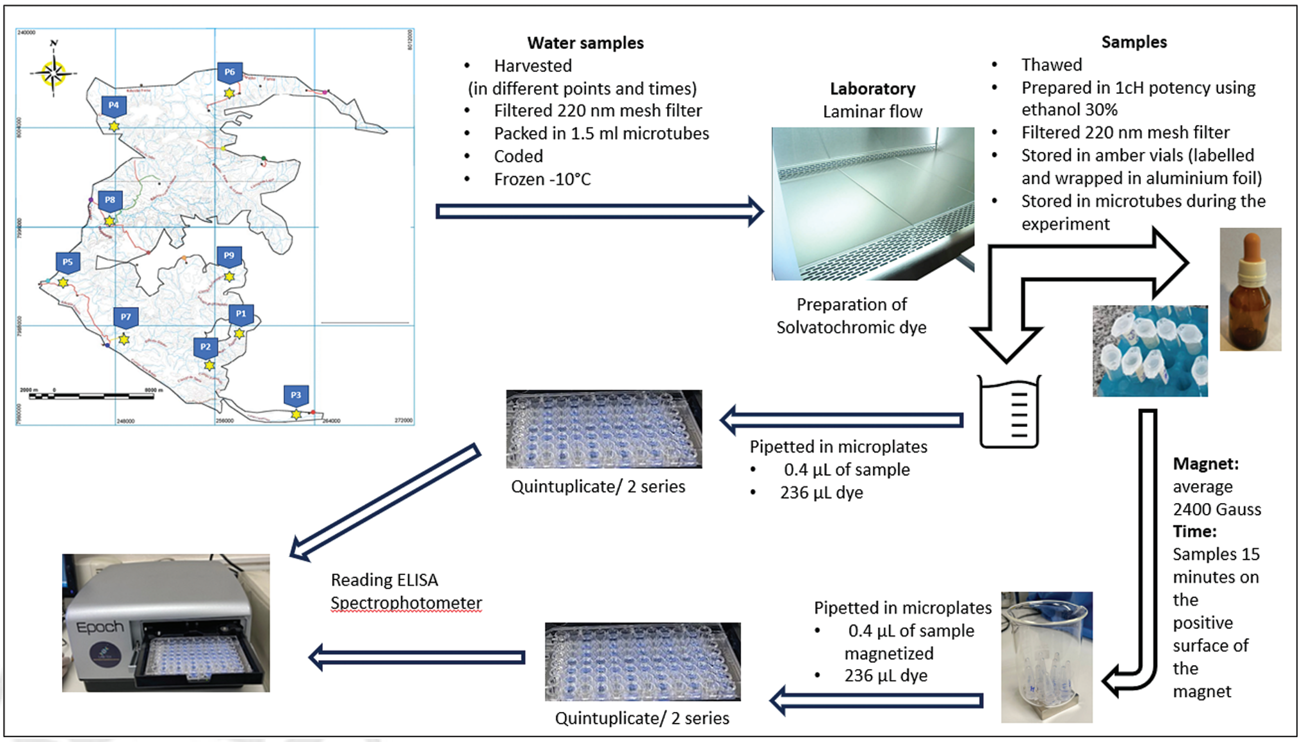

Fig. 2 Experimental design for the analysis of the water samples. Version of map adapted by permission of the original author.[47]

to prevent any alteration of the dyes, which are sensitive to light. The reading was done in a spectrophotometer with millesimal sensitivity and a scan of at least 1 nanometer (FEMTO 800 XI, São Paulo, Brazil). Readings proceeded just after the preparation of each cuvette set, avoiding alcohol evaporation.

A bucket with PA ethanol (Synth, Diadema, Brazil) was initially read for each dye to calibrate the equipment. Then, each pure dye was serially read to determine the absorbance peak and the respective wavelength. The reading was done by scanning the visible light spectrum, whose wavelengths vary between 350 and 800 nm, and the absorbance of each one was noted.

Water Sample Analyses

After the preliminary screen described above, methylene violet was selected as a marker to test the water samples. The microplate/ELISA (enzyme-linked immunosorbent assay) reader protocol was used in this case to allow the reading of multiple samples at the same time, as previously described.[1,9] Water at pH 4.0 was chosen as the solvent for methylene violet to set the best sensitivity of this dye.[14–16] Previous studies corroborate this choice, as does the relative equivalence seen between water at pH 4.0 and ethanol when both are used as solvents for methylene violet.[42]

The 10X stock solution of the dye was prepared in autoclaved water containing 0.2% DMSO (dimethyl-sulfoxide, SIGMA-MERCK, Saint Louis, Missouri, United States) and filtered through a 0.22-µm mesh filter, obtaining a final concentration of 500 µM. Then, the pH adjustment to 4.0 was made with a pH meter (KASVI K39–2014B, São José dos Pinhais, Brazil) by adding one or two drops of 0.1 M citric acid solution. The stock solution was kept at 4°C in a refrigerator.

On the day of the experiment, the stock solution was diluted in sterile distilled water at a 1:10 ratio to obtain the final concentration of 50 µM, and the pH was adjusted again. After preparation, the dye was filtered with a 0.22-µm mesh filter and stored in a Falcon flask wrapped in aluminium foil to protect the dye from light. For each round, a new batch of dye was prepared.

The water samples were analyzed in microplates (COSTAR, Davis, California, United States), where 4 µL of sample and 236 µL of dye were added to each well and read in an ELISA spectrophotometer (Epoch, BioTek, Agilent, Santa Clara, California, United States).

As described above, in all cases the samples underwent 30 additional manual succussions mimicking the Denise device mechanical arm movements immediately before being filtered with a 0.22-µm mesh filter and placed in 2.0-mL microtubes until the moment of their use.

The Sample Magnetization Step

A supplementary method was adopted to optimize the identification of homeopathic potencies through interaction with solvatochromic dyes. Thus, the following step was performed in two ways (►**Fig. 2**): simply reading samples in the ELISA reader, or submitting the samples to a static unidirectional strong magnetic field prior to reading in the ELISA reader. The second approach was based on literature evidence about magnetic fields' effects on polar solvents.[43–46]

In the latter case, samples contained in microtubes were placed inside a beaker and positioned on the positive surface of a neodymium (NdFeB) magnet model N52, 240mT/2400G average, measuring 5.08 × 5.08 cm × 1.27 cm (Magnetum Produtos Magnéticos, São Paulo, Brazil), for 15 minutes.[1,9] Then, the samples were immediately pipetted into the microplates, and the reading was done in an ELISA spectrophotometer (Epoch, BioTek, Agilent, Santa Clara, California, United States). The samples were analyzed in quintuplicate in two experimental replications, resulting in $N = 10$. The controls were the FPT vehicle (30% non-successed ethanol) and purified water, which were used to check for any non-specific effects of magnetization.

The dyes and samples were manipulated in a sterile environment, with temperature, humidity, and background magnetic flux controlled and recorded (temperature 25.5 ± 1.31°C, humidity 34.5 ± 12%, magnetic field 0.13 ± 0.06 µT). Measurements were made with a thermohygrometer (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). All materials were autoclaved or cleaned with 70% alcohol and left in the laminar flow under UV light for 15 minutes before use. The dyes and samples were placed in the laminar flow after extinguishing the UV light to avoid material alteration.

Before proceeding with the serial evaluation of magnetized water samples, a pilot study was conducted to test a putative non-specific effect of magnetized water itself.

Correlating Tracking with Environmental Features of the State Park

Since the identification of FPT activity varied as a function of time, it was possible to establish four response patterns (none, early, late, and constant signaling). Thus, the following step of data analysis was to verify if there were any correlations between these patterns and relevant data about levels of environmental vulnerability of the areas near the intervention points. Correlations between water samples' response to methylene violet (signal maintenance) and levels of environmental vulnerability to fire were calculated. These levels have previously been described for PENT[47,48]:

- Relative hazard vulnerability identifies polluting enterprises or other economic activities that may pose a risk to the park integrity as a function of their proximity to each evaluated area.
- Environmental vulnerability identifies degrees of vulnerability according to slope and its capacity to propagate fire.
- Fuel vulnerability identifies the type of stratum (vegetal covering) and the amount of biomass in each region, leading to a higher or lower susceptibility to burn.
- Multi-temporal analysis identifies different levels of forest preservation/regeneration from 1985 to 2004, resulting in areas more or less vulnerable to fire.
- Natural vulnerability identifies degrees of vulnerability according to the landform and its capacity to propagate fire.

A short description of each vulnerability type, its given score, and the corresponding intervention points is presented in ►**Table 2**.

Statistical Analysis

Values used from dye and sample assays in the statistical analysis were the delta absorbance (absorbance of each sample minus that of the dye itself).

For the statistical analysis, the Windows Prism 10.0 program was used. Data were checked for Normality by the Shapiro–Wilk test, and deviations were normalized by Q–Q plot inspection. One-way analysis of variance followed by the Tukey method was performed to evaluate absorbance differences among samples in the solvatochromic dye method. The Tukey boxplot mode in Prism 10.0 software automatically identified outliers to be removed. For variables that did not comply with Normality, the non-parametric Kruskal–Wallis/Dunn method was applied.

The correlation matrix and Spearman r test were employed to evaluate any correlation between the scores of dye responsivity to samples harvested from each intervention point, as measured, and the levels of relative environmental vulnerability, as previously defined.[47]

Values of $p \leq 0.05$ were considered statistically significant.

Results

Selection of Dyes

Among the six dyes tested, methylene violet was found to be the most suitable marker for FPT, because it gave the greatest statistically significant results when the optical density delta between FPT and 30% non-successed alcohol was assessed (►**Fig. 3** and ►**Supplementary Fig. S2**, available in online version only). Similar results were obtained when this dye was tested using either the cuvette/spectrophotometer or the microplate/ELISA reader method (►**Supplementary Fig. S2**, available in online version only). Changes in the delta absorbance of methylene violet (positive or negative) indicate the presence of FPT in water samples.

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

The analysis of water samples with methylene violet followed previous testing of FPT in microplates using the protocol involving static magnetic treatment outlined in Materials and Methods. Dye delta absorbances using magnet-treated FPT samples were statistically significant ($p = 0.0045$) in relation to magnet-treated vehicle (30% alcohol). Magnet-treated water did not differ from the magnet-treated vehicle but presented a higher variance than FPT, indicating the absence of non-specific results related to magnet-treated water itself (►**Supplementary Fig. S3**, available in online version only).

Two series of experiments were run to track FPT, using magnet-treated and non-magnet-treated water samples taken from watercourses. In the non-magnet-treated samples, results showed only three points presenting significant trackable changes (P2, P7, P9), indicating FPT signals in the watercourse system, with changes occurring later for P7 and P9 than for P2 (►**Fig. 4**). On the other hand, the magnet-treated samples revealed clearer differences (►**Fig. 5**).

Table 2 Categories and scores of environmental vulnerability areas of the PENT[47,48] and the corresponding intervention points

Vulnerability category	Descriptive level [47]	Given score	Intervention points
Relative hazard vulnerability (industry, rural area, lack of sanitation control, mineral extraction)	Indirect influence zone (5,000 m from the park area)	1	P1, P3
	Direct influence zone (2,000 m from the park area)	2	P9
	Roads close to the park area	3	P5, P6, P7, P8
	Impact zone (400 m from the park area) (►**Supplementary Fig. S1**, available in online version only)	4	P2, P4
Environmental vulnerability	Very low	0	–
	Low	1	–
	Medium	2	P1, P2, P5, P6, P7, P8, P9
	High	3	P3
	Very high	4	P4
Fuel vulnerability	*Cerradão/Mata* (high-density savannah)	0	P1, P2, P4, P5, P7, P8
	Cerrado (savannah)	1	–
	Cerrado ralo (low-density savannah)	2	–
	Campo sujo	3	P3, P5, P6, P7, P8, P9
	Campo limpo	4	–
	Cambaúva	5	–
Multi-temporal analysis	Native vegetation	0	P1, P2, P3
	Regenerated native vegetation	1	P4
	Anthropic/agriculture area	2	P5, P6, P7, P8, P9
Natural vulnerability	Very low	0	–
	Low	1	P6, P7
	Medium	2	P3, P5
	High	3	P4, P8, P9
	Very high	4	P1, P2

Abbreviation: PENT, *Parque Estadual das Nascentes do Rio Taquari*.
Note: The signal maintenance and the corresponding points were classified as:
1- No signal—score 0—P2, P4.
2- Early signaling—score 1—P5, P6, P8.
3- Late signaling—score 2—P1, P9.
4- Constant signaling—score 3—P3, P7.

Tracking of the FPT signals using samples subjected to the static magnetic field indicated four response patterns: no response (P2, P4), early transitory response (P5, P6, P8), late response (P1, P9), and constant response (P3, P7). Consequently, prior exposure of water samples to a static magnetic field was considered the best protocol to use.

After performing the magnet/microplate/ELISA spectrophotometer protocol, differences in delta absorbances of water samples harvested before and after the insertion of the homeopathic complex through time were apparent in seven of the nine points (P1, P3, P5, P6, P7, P8, P9). In points P3 and P7, delta absorbance was constant over time. This contrasted with points P5, P6 and P8, where the absorbance varied, being higher in the first hours but decreasing later (early transitory response). For P1 and P9, absorbance increased only after 72 hours (late response).

At points P2 and P4, there was no significant change in delta absorbance over time (►**Fig. 5**). To understand this result, an analysis of the park's management plan[47,48] was performed, in which a scoring system was created to ascertain any possible correlations between environmental factors and the traceability of FPT in water samples. With this information, a multi-factorial statistical analysis (correlation matrix with Spearman *r* test) was made, resulting in ►**Fig. 6**. These results showed a correlation between anthropic activity at less than 400 m from points P2 and P4 and the respective lack of dye delta absorbances, with $p = 0.013$. According to Carrijo,[47] the anthropic activity at these

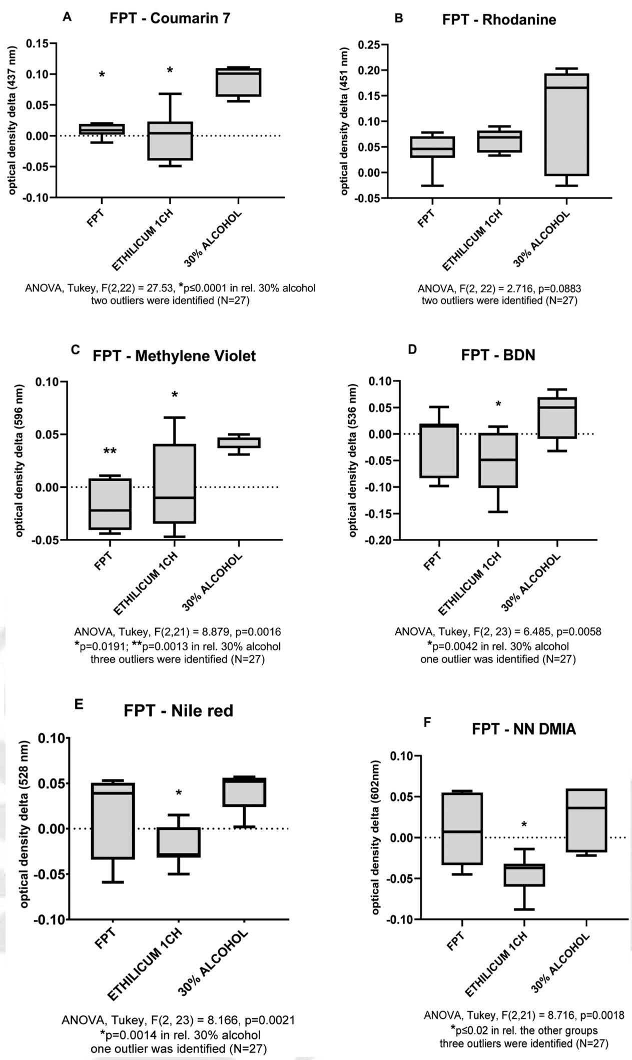

Fig. 3 Screening of solvatochromic dyes to identify the formulated homeopathic complex (*Formula Parque Taquari*—FPT). The statistical data are represented in each graph. Statistical significance is represented by *.

locations were mineral extraction (removal of gravel pit) near point 2, and small farms near point 4 (►**Table 2**, ►**Supplementary Fig. S1**, available in online version only).

Discussion

The present study aimed to evaluate the possibility of tracking homeopathic preparations from water samples harvested in different areas of the large water source and course system of PENT after it suffered a devastating fire in 2020, and subsequently to perform a statistical correlation between the presence or absence of water–dye interaction and relevant environmental features at each intervention point. A homeopathic complex was designed specifically for mitigating the fire damage to the local diverse flora and fauna and was applied at nine strategic points in the watercourses.

The results can be listed as follows: (1) methylene violet was found to be the most suitable dye for identifying and tracking the homeopathic complex FPT through the watercourse system; (2) subjecting samples to a static magnetic field combined with ELISA reader using microplates showed high sensitivity and effectiveness in tracking the homeopathic complex in water over time; (3) four tracking patterns were described: no response (locations P2, P4), early transitory response (P5, P6, P8), late response (P1, P9), and constant response (P3, P7); (4) the samples from locations P2 and P4, which could not be tracked, were statistically correlated with permanent anthropic interventions near those areas.

It is not entirely clear why methylene violet was the most suitable dye for tracking the FPT complex out of the six dyes tested. The sensitivity of solvatochromic dyes to homeopathic potencies might depend on several factors related to quantum electrodynamics (QED), including the dye's ground state dipole moment, its molecular rigidity, and conformational differences between a dye's ground and excited states.[16] Some or all of these factors may be at play in the current study. The role of bubbles and cavitation must also be considered in further studies using specific and sensitive techniques such as dynamic light scattering and near-infrared spectroscopy.[13,49,50]

QED postulates that a coherent system is characterized by a single rhythmic oscillation, in which all the components of the quantum system are correlated over long distances, thereby allowing the transition from a quantum scale up to a macroscopic one. According to QED, it is postulated that energy can travel in a coherent medium in the form of *solitons* without any losses.[51,52]

Using solvatochromic dyes to track homeopathic medicines[14–16] is based on their ability to respond to several environmental conditions and agents, including solvent polarity and ambient electric fields,[18] even when samples are highly frozen and then thawed,[2,9,17] as shown in similar models.[53]

It should be noted that homeopathic complexes behave as unique medicines from the physicochemical point of view.[54] Thus, their interaction with the dyes would be expected to be similar to that of homeopathic medicines made from a single substance. Herein, the clear responsivity of the FPT complex to methylene violet using different solvents and devices (►**Supplementary Figs. S2** and **S3**, available in online version only) corroborates this finding.

Prior to analyzing watercourse samples, a series of experiments was performed to establish the best protocol for tracking FPT signals in the water samples following the insertion of the devices containing the homeopathic complex. The cuvette method was first used to read the samples in a spectrophotometer. This method was useful for selecting dyes since it can run the full visible spectra in a few minutes. However, the microplate/ELISA reader protocol is the best option in cases of processing a higher number of samples since they are read simultaneously, reducing variance between one and another and making the method more accurate.

In previous studies, we observed that detecting homeopathic potencies in heterogeneous liquid media[6,9] using solvatochromic dyes presented greater variance in the analyses compared with pure medicines produced in hydroalcoholic solution in a standardized manner.[9,27,28] It is speculated that the heterogeneity of water samples obtained

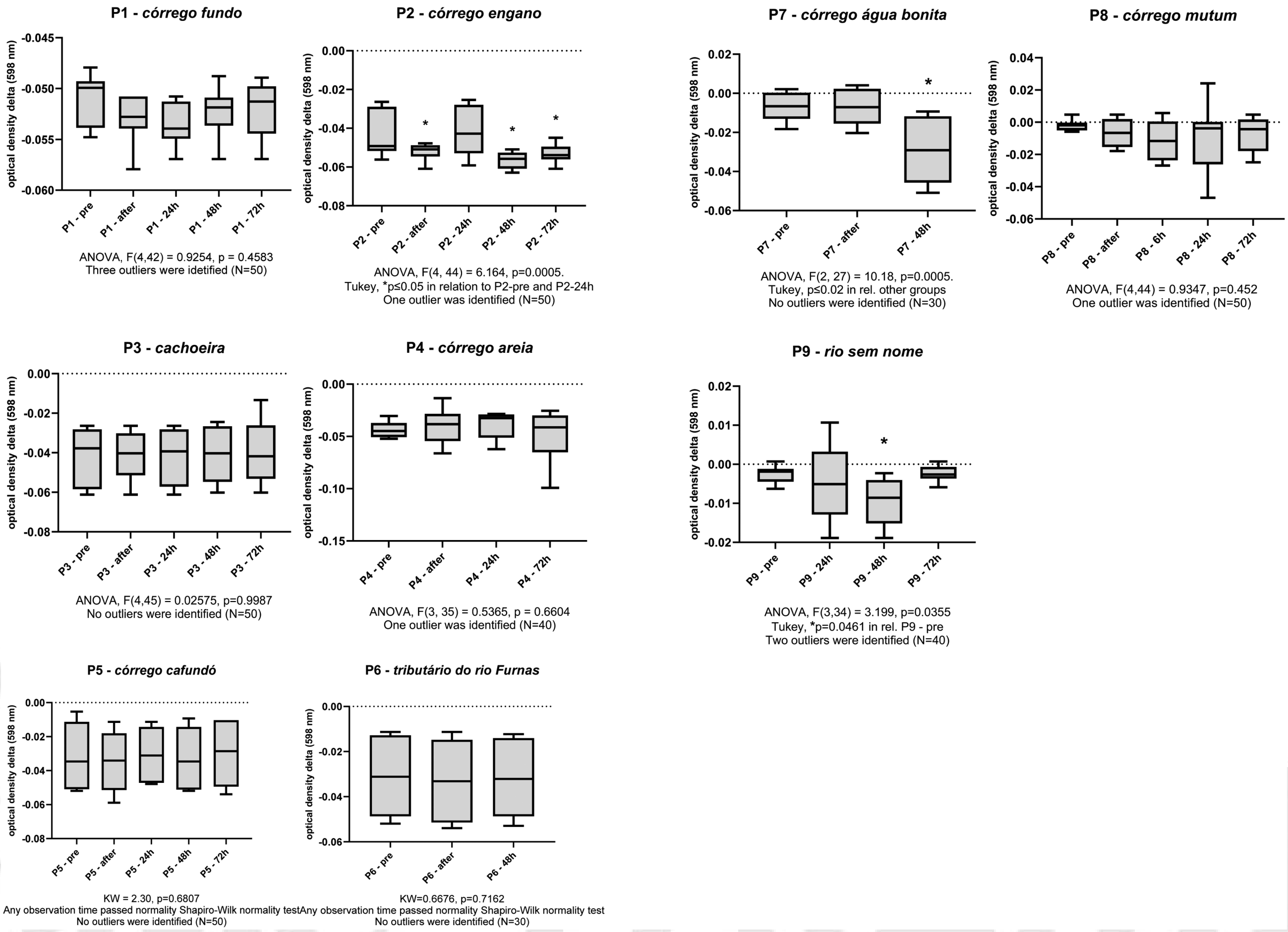

Fig. 4 Optical density delta as a function of the time at different device installation and water sample harvesting points, using solvatochromic dye methylene violet (ELISA reader/microplate protocol). Samples were analyzed *in natura* (with no magnetization). FPT, *Formula Parque Taquari*—homeopathic complex; PRE, just before the intervention. The names of the points/watercourses (P1–P9) are designated by their proper names in Portuguese. The statistical data are represented at the bottom of each graph. $N = 10$ for each group.

from natural watercourses produces signal "noise" through non-specific interactions with the dyes.[42] Adequate dilution of water samples prior to assay was found to abolish these non-specific interactions.

It was postulated that prior subjection of samples to a static magnetic field might increase the sensitivity of the method by ordering water along the magnetic flux lines produced by a strong magnet, thereby potentially stabilizing and/or enhancing the homeopathic signal, which could be easily identified by methylene violet dye due to its high sensitivity to the solvent's photophysical properties.[55] This postulate was suggested by previous observations[56] and supported by the results obtained by Mohammad and colleagues.[9] The magnetic field intensity used is within the range employed in nuclear magnetic resonance spectroscopy, a technique that has been shown capable of identifying homeopathic potencies in a number of studies.[10]

Using methylene violet as the most suitable dye for tracking the FPT complex and the protocol of subjecting samples to static magnet fields as a standard procedure made possible comparisons between the points in the park chosen for device placement. Results not only showed that the FPT complex could be tracked through the watercourses but also suggested environmental factors interfering with the tracking process at points P2 and P4 (►**Table 2**; ►**Supplementary Fig. S1**, available in online version only). The charts presented by Carrijo[47] and by the IMASUL report in 2018[48] show the contemporaneity of many of these environmental factors with fire events. Thus, the quality and homogeneity of the water samples could explain the variations in FPT tracking at different points.

Critical points such as mining areas for the removal of a gravel pit are shown in various segments of the park classified as highly vulnerable to relative hazard,[47,48] including areas near location P2—"*córrego Engano*"—where there is also, in the vicinity, a viewpoint and a tourist reception structure.[57] Likewise, location P4—"*córrego Areia*"—is in another highly vulnerable zone due to a steep slope with rugged area on the edges of plateaus subject to surface runoff, together with small-farm activity and lack of proper garbage collecting.[47,48] Both P2 and P4 are in the so-called impact zones (►**Table 2**).

The correspondence between the location of these points and the absence of methylene violet dye interaction with the respective water samples was statistically shown by the Spearman *r* test (►**Fig. 6**), which suggests that anthropic

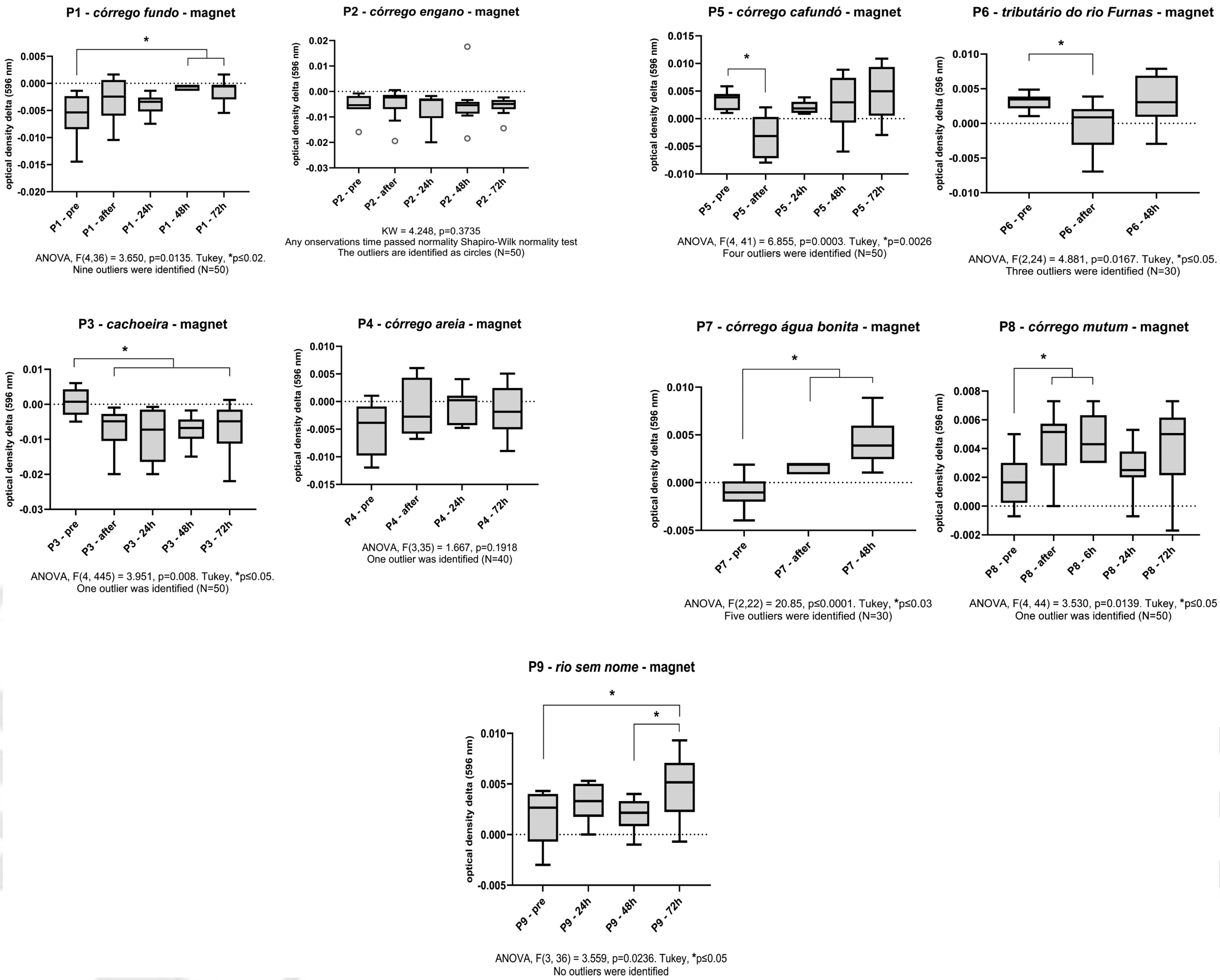

Fig. 5 Optical density delta as a function of the time at different device installation and water sample harvesting points, using solvatochromic dye methylene violet (ELISA reader/microplate protocol). Samples were subjected to a static magnetic field of 2400 Gauss for 15 minutes before reading. FPT, *Formula Parque Taquari*—homeopathic complex; PRE, just before the intervention. The names of the points/watercourses (P1–P9) are designated by their proper names in Portuguese. The statistical data are represented at the bottom of each graph. $N = 10$ for each group.

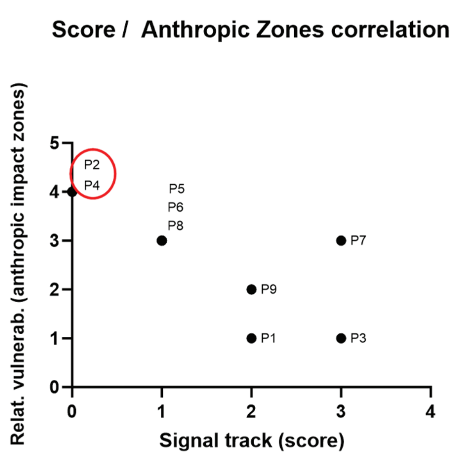

Fig. 6 Graphic representation of the Spearman *r* test details. This test was employed to evaluate the correlation between the Signal track score and the levels of relative environmental risks (anthropic impact) of PENT. Note that P2 and P4 did not present interaction with methylene violet (signal track) but showed the highest anthropic impact score, whose correlation yielded $p = 0.013$.

factors could be an important element for the non-permanence of homeopathic signaling in these ecosystems. In contrast, the type of biome, vegetation, and soil does not seem to interfere in homeopathic signaling since there was no statistical correlation between these factors and the type of response of water samples to methylene violet.

More research is needed to understand this phenomenon, preferably studies in which a reference area could be used as a control, allowing a cause–effect conclusion. Only an association between variables can be inferred from the current work. The possibility of other, yet unknown, environmental variables determining early or late response to the dyes and their putative relation with biological outcomes is still an issue that needs to be explored in further investigations.

Conclusion

Among the six dyes tested, methylene violet was the most suitable tracking dye for the homeopathic complex used in the PENT State Park. Subjecting samples to prior static magnetic field treatment and reading in an ELISA spectrophotometer using microplates increased the method's sensitivity. It allowed the processing of many samples at the same time, making tracking the FPT signal in the watercourses possible. The homeopathic complex could not be tracked at locations where there was significant anthropic impact, suggesting that varying environmental aspects must be considered when this methodological approach is applied in field conditions.

Highlights

- Solvatochromic dyes are able to track homeopathic preparations even in large volumes of water obtained from natural environments.
- Methylene violet was found to be the most suitable dye to track a homeopathic complex formulation in the watercourses of a fire-damaged state park.
- Submitting samples to an average static and unidirectional magnetic field of 2400 Gauss improved the detection method's sensitivity.
- Environmental disturbance due to anthropic impact may interfere with the traceability of homeopathic preparations using the solvatochromic dye method.

Supplementary Material

Supplementary Fig. S1. Vulnerability integrated zoning of PENT.
Supplementary Fig. S2. Comparison of cuvette and microplate reading methods.
Supplementary Fig. S3. Controls comparison.

Author Contributions

N.S.S.M.: Main researcher, postgraduate student, involved in all experimental procedures, writing. A.A.G.P.: Experimental procedures—solvatochromic dyes tests. S.F.: Experimental procedures—solvatochromic dyes tests. I.B.S.: Experimental procedures—solvatochromic dyes tests, statistical analysis. M.F.S. and A.P.F.: Design, production, and distribution of homeopathic preparation. S.J.C.: Experimental design with solvatochromic dyes. L.V.B.: Main adviser, coordination of all steps of the study, discussion of results, writing.

Conflict of Interest

Sigo Homeopatia company donated the water samples in partnership with the governmental organization IMASUL. The researchers were not given any other resources from either organization.

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