DEPLOYMENT OF INDEPENDENT METHOD OF CULTURE TO DETECT BACTERIA AND ARCHAEA DOMAINS IN DRAINAGE BASIN UNDER THE INFLUENCE OF URANIUM MINING – ORE TREATMENT UNIT, CALDAS/MG

Palvo J. Borba Junior[1,2], Heliana de Azevedo[2] and Leilane Barbosa Ronqui[2]

[1] Universidade Federal de Alfenas – UNIFAL
[2] Comissão Nacional de Energia Nuclear – CNEN - Laboratório de Poços de Caldas – LAPOC

jouber_borba@hotmail.com, hazevedo@cnen.gov.br and leilanebio@yahoo.com.br

The Ministry of Science, Technology and Innovation

INTRODUCTION

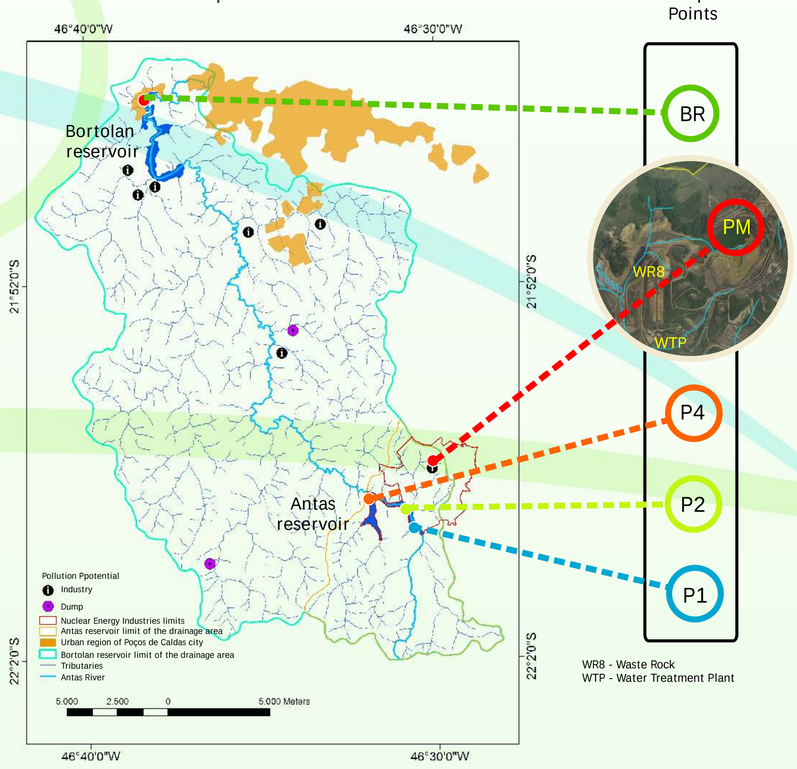
In the Ribeirão das Antas Hydrographic Sub-Basin region in Poços de Caldas/MG, it is possible to find aquatic environments with these characteristics, where potential environmental impacts have been evidenced due to anthropogenic actions from industries located in the vicinity of the reservoirs of this sub-basin, such as mining activities, fertilizer production, food industries, fish farming, among other activities that lead to the silting of dams and streams (RONQUI, 2008).

There are two reservoirs that stand out in the ecological ambit when it comes to this type of occurrence. The first aquatic body, where the sub-basin begins, is Antas reservoir (AR), a dam which receives treated effluents from the Brazilian Nuclear Industries - Ore Treatment Unit (BNI-OTU), where is localized the Pit Mine - PM), characterized by the presence of high concentrations of heavy metals and radioactive chemical species, as well as low pH values (NASCIMENTO, 1988 and NÓBREGA et. al 2008). The second studied environment is another dam – Bortolan reservoir (BR) – which receives waste discharges from industries and residences in the vicinities.

For the identification and analysis of the microorganisms was used method independent method of cultivation FISH (*Fluorescent In Situ Hybridization*) (WAGNER et al., 2003), also analyzed the physical and chemical variables of water samples in order to characterize the system.

METHODOLOGY

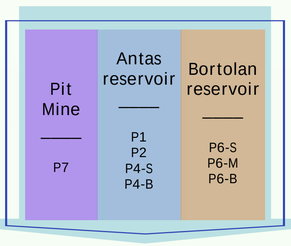
Sample Points



WR8 - Waste Rock
WTP - Water Treatment Plant

Sample Points

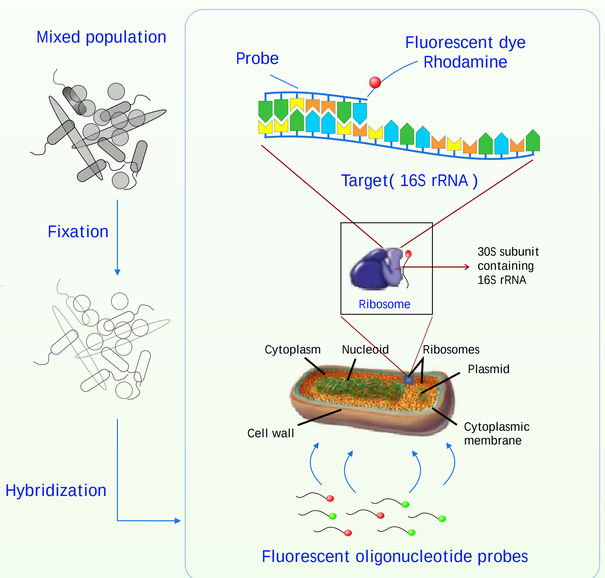
Water sample

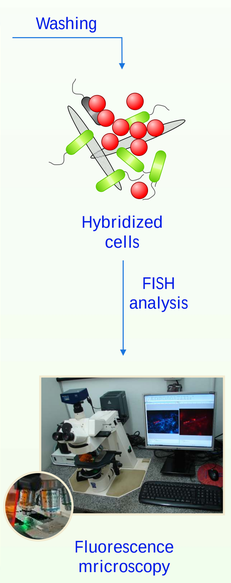


Physical and Chemical Parameters

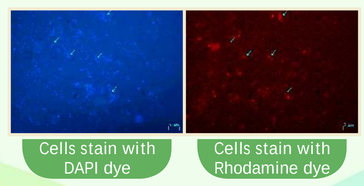
- Temperature
- Dissolved Oxygen
- Transparency
- collection Depth
- pH
- Conductivity
- Nitrite
- Nitrate
- Total Nitrogen
- Ammonia
- Total phosphorus
- Dissolved phosphate
- Inorganic phosphate
- Sulfate
- Fluoride
- Manganese
- Zinc
- Uranium
- Thorium

Fluorescent *in situ* Hybridization





The FISH method was adapted by AMANN, 1994; AMANN et al, 1994 and AMANN et al, 1995. EUB338 probes for the Bacteria domain and ARC915 for Archaea, both stained with Rhodamine were used, as well as polycarbonate membranes of 0.22 um, washing and hybridization buffers with variable concentrations of formamide and sodium chloride, paraformaldehyde (PFA - 4%), in addition to laboratory equipment and the glassware necessary for the method implementation.



Cells stain with DAPI dye | Cells stain with Rhodamine dye

RESULTS

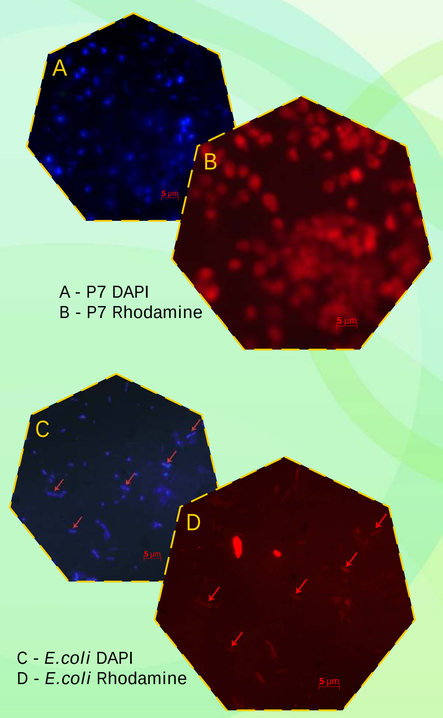
Average values of physical and chemical variables in water samples from Antas and Bortolan reservoirs and the Osamu Utsumi Pit Mine (BNI-OTU)

Variables	AR	BR	PM	CONAMA	COPAN	CNEN
Temperature (ºC)	21.08	21.43	21.93	< 40	< 40	-
Dissolved Oxygen (mg.L^{-1})	6.83	6.96	6.57	> 5.0	> 5.0	-
Transparency (m)	1.52	1.25	-	-	-	-
collection Depth (m)	0.5 – 6.9	0.5 – 6.0	0.10	-	-	-
pH	6.54	6.81	3.84	6.0 – 9.0	6.0 – 9.0	-
Conductivity (µS.cm^{-1})	298.50	78.73	1347.38	-	-	-
Nitrite (µg.L^{-1})	0.60	2.04	1.23	< 1000	< 1000	-
Nitrate (µg.L^{-1})	58.53	174.59	28.70	< 10000	< 10000	-
Total Nitrogen (µg.L^{-1})	860.71	701.39	735.00	-	-	-
Ammonia (µg.L^{-1})	17.84	64.46	6.50	-	-	-
Total phosphorus (µg.L^{-1})	8.34	23.85	4.48	< 50.00	< 50	-
Dissolved phosphate (µg.L^{-1})	1.77	4.80	1.03	-	-	-
Inorganic phosphate (µg.L^{-1})	0.49	1.92	1.46	-	-	-
Sulfate (mg.L^{-1})	92.81	26.47	712.74	250	250	-
Fluoride (mg.L^{-1})	1.45	0.50	39.5	1.4	1.4	-
Manganese (mg.L^{-1})	0.60	0.31	71.4	0.10	0.10	-
Zinc (mg.L^{-1})	0.03	0.02	9.95	0.18	0.18	-
Uranium (mg.L^{-1})	< 0.02	< 0.02	2.31	0.02	0.02	0.01
Thorium (mg.L^{-1})	0.02	0.02	0.09	-	-	0.07

- it does not exist

Tests for the standardization of the independent culture technique of FISH, conducted with water samples from Antas and Bortolan reservoirs and Osamu Utsumi Pit Mine

Points	Probes	Probe / hybridization buffer (µL/µL)	Formamide [ ] (%)	NaCl wash buffer (mM)	Hybridization time (h)	Hybridization temperature (ºC)
P1	EUB338	3/16; 5/70	0; 5	225	1.5; 2.0; 2.5	46; 48
P2		6/32	30		1.5; 2.0	46
P7		3/16	20		2.5	46
P4S		6/32	20; 30		2.0	46; 50
P4M		3/16	20		2.5	46
P4F		6/32; 7/40; 8/50	20; 30		1.5; 2.0	46; 48; 52
P6S		3/16; 6/32; 7/40	20; 30		1.5; 1.75; 2.0	46
P6M		3/16	20		2.5	46
P6F		6/32; 7/40; 8/50	20		2.0; 3.0	46; 48; 50; 52
P1	ARC915	6/32; 5/70	0; 5; 20	225	1.5; 2.0	46; 48;
P2		x	x		x	x
P7		x	x		x	x
P4S		6/32	20		2.0	46
P4M		x	x		x	x
P4F		x	x		x	x
P6S		x	x		x	x
P6M		x	x		x	x
P6F		6/32	20		2.0	46



A - P7 DAPI
B - P7 Rhodamine

C - *E.coli* DAPI
D - *E.coli* Rhodamine

Images captured by the camera attached to the epifluorescence microscope

CONCLUSION

We can conclude that bacterial cells are present in water samples from the environments studied. However, due to unsatisfactory results further changes will be conducted based on new information obtained through the literature and direct communication with researchers experienced in this methodology that are cited in the literature. Therefore, further tests will be conducted, aiming to standardize a FISH protocol for Bortolan and Antas reservoirs and the Osamu Utsumi Pit Mine. These tests will continue to be based on existing literature, seeking future comparisons to aquatic environments that may present similar characteristics.

SUPPORT

USP FAPEMIG CNEN Brazilian Nuclear Energy Commission

Brazilian Nuclear Energy Commission - CNEN
Laboratory of Poços de Caldas - LAPOC
P.O. Box. 913 - Poços de Caldas, MG - Brazil
lapoc@cnen.gov.br