

# Surface Geochemical Exploration Using Bacterial and Plant Bioindicators, Northern Neuquen Basin, Argentina\*

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## Abstract

Organotroph bacterial accumulation and trace elements present in soils and sediments are good indicators for active oil reservoirs due to microseepage of light hydrocarbon gases and other geogases that reach the surface through pore spaces or fracture systems at both local and crustal scales. Trace elements like V, Cr, Ni, Mo among others, are also carried upwards in the shape of microbubbles by means of diffusion and hydrothermal activity. The surface and subsurface alteration of sediments and soils is therefore the expression of the ascending gas plumes.

In northern Neuquén Basin, Cerro de Los Leones area, a geochemical exploration survey took place based on transects with sampling points of 400 meters distance from one another. Transects were perpendicular to the structures and faults present in the area to show the contrast between anomalies and background values. Geological formations were taken into account when sampling the soil surface, identifying rock types, development and origin. Satellite image processing with NDVI and Tasseled Cap algorithms were used to discriminate plant photosynthetic efficiency and soil moisture. Soil samples for microbial analysis were taken at 40 cm depth and placed in sterilized jars. Using a modified MPOG method, edaphic bacterial colonies were counted in UCF/g. Also samples were taken from the same depth for trace element analysis using X-ray fluorescence. Plant community analysis involving Shannon Diversity index (H) and Specific Richness index (S) was also carried out to show the local effect of soil alteration in vegetation. Square sample stations (6 meters side) were designed for each sample point of one transect. A geomorphological feature such as soil salinity was taken into account to correct the results of plant indexes and better show the effect of microseeps on the ecosystem. Every result was integrated to seismic information to construct the possible arrangement of the petroleum system. This research helped to identify active reservoirs through permeable faults and structures.

The study area is located southeast of Malargüe City near the Llanquanello Lagoon ([Figure 1](#) and [Figure 2](#)). The geologic column belongs to the Mendoza sector of the Neuquén Basin. The oil system is composed of Jurassic and Early Cretaceous source rocks (Molles, Vaca Muerta and Agrio formations), while Cretaceous and Tertiary rocks are the reservoirs of the system.

### **Biogeochemical Model**

The study of organisms such as plants and microbes that inhabit the soils of oil fields proved to be an interesting complementary tool to understand and analyze the dynamics of biogeochemical events that take place in those environments (Larriestra et al., 2012). There is a close relationship between microbes, vascular plants, soils and microseeping gases from underground hydrocarbon reservoirs containing methane, ethane, propane or butane, that reach the surface via the sediment pore spaces, fractures and faults. These migrations produce particular conditions near surface soil which favors the growth of organotroph microorganisms which exploit these energy resources to maintain their subsistence requirements. The metabolic activity of these microbes produce nutrient solution consumption, increased pH, decreased Eh, low oxygen pressure and precipitation of salts (Schumacher, 1996; Saunders et al., 1999).

These factors influence the physiology of plants, producing different types of expression such as anomalous distribution patterns, different levels of vegetation cover, certain values of species diversity, growth and development disorders, etc. (Vagney et al., 2005; Larriestra et al., 2009). The gas plume that changes the ecosystem's natural dynamics is detailed in [Figure 3](#).

Trace elements as V, Cr, Mn, Ni, As, Co, Cu, Mo, U, Fe, Zn and Pb have been documented forming halos in association with hydrocarbon reservoirs and oils (Leaver and Thomasson, 2002; Marakushev and Marakushev, 2006).

These trace elements may be the residual product of bacterial activity in the oxidation of hydrocarbons or come directly from the reservoir migrating through the bedrock in complex association with hydrocarbon microbubbles. Furthermore, they may be dragged to the surface by geogases (Sikka and Shives, 2002) from the mantle passing through the reservoir and leaving imprints as differential concentrations of trace elements on their surface.

The presence of mineral halos may or may not be related to recent bacterial activity, since the conditions that determine the precipitation of minerals related to reducing gases feathers migrants, is an event that may have taken place in the geological past. The existence of fractures and/or faults greatly generates surface expressions of microleakages, leading to temporary local anomalies that must be distinguished from the “paleoanomalies” (Larriestra et al., 2010a) produced along the history of the reservoir ([Figure 4](#)).

### **Methodology**

A transect comprising 57 sample stations were made with 400 meters spacing between them. At each sample station, soil samples were taken for X-ray fluorescence and butane-trophic bacteria analysis. These samples were taken from a depth of 20 to 40 cm depending on the soil characteristics and put on sterilized jars for bacteria and in hermetic sachets for trace elements. Also, plant species were recorded for diversity and richness analysis.

## **Microbiological Analysis**

This analysis is performed according to the method MPOG-Microbial Prospecting for Oil and Gas, (according to Rasheed et al., 2008, and Wagner et al., 2002) with some modifications (Larriestra et al., 2010b). For their development, soil microorganisms have certain requirements such as temperature or the presence/absence of oxygen. Within these requirements, some of which are common to all organisms such as the need to oxidize carbon sources for the formation of biomolecules and cell structures.

In the present analysis, a minimal medium is used containing all sorts of nutrients to allow the development of the cells, but free of carbon sources. The goal is to affect the flora present in the soil with an enriched butane atmosphere so those capable of using butane as a carbon source to perform vital functions remain viable.

This method is based on the counting bacteria present in the sample that were able to develop in the specific conditions of the study. Furthermore, samples are filtered for all sources of carbon to avoid false positives. By default, the counting range of the method is between  $1 \times 10^2$  and  $1.8 \times 10^6$  CFU/g.

## **Trace Element Analysis**

The record of major and minor trace elements on soil samples was done by using a portable X-Ray Fluorescence device that shows 32 chemical elements (ppm units) that could be located on the surface due to the effect of microseep geogases coming from the reservoir or the mantle. The analysis involved the exposure of the sample (previously dried in a warm oven) for 2 minutes to X-rays. Also, trace element analysis of the oil was carried out using atomic absorption spectrometry (AAS) to search for a relationship between the metals present in the oil and at the surface.

## **Plant Community Analysis**

For the analysis of vascular plant communities, sampling stations of 6 square meters were made at the same point from which soil samples were previously obtained for bacteria and trace elements. Based on the characteristics of the flora, the study comprised (based on Magurran, 2004):

- Shannon Diversity Index (H).
- Specific Richness (total number of species).
- Identification of a possible bio-indicator plant species.

## Digital Satellite Data Processing (NDVI) Analysis for Soil and Vegetation

The Normalized Difference Vegetation Index (NDVI) is a digital satellite processing that uses the relationship between red and near infrared wave spectra to show the vegetation photosynthetic activity, the biomass production and plant cover. It ranges between 0 for bare soil and 1 for full plant cover (Paruelo et al., 1998).

Finally, data integration with seismic data, bacteria UFC/g, trace elements and plant community anomalies were performed to verify the biogeochemical model.

## Results

According to the results of bacteria and trace elements present in the soils and plants, there is evidence of light hydrocarbons such as butane and other carrier gases reaching the soil surface.

Butane trophic bacteria showed 3 high peaks of UFC/g ([Figure 6](#)). Two of them were located in the center, over well-defined faults and a third one located in the eastern side of the transect, having no structural information available for this group of samples ([Figure 5](#)).

Soil trace elements such as vanadium, barium and zinc ([Figure 8](#), [Figure 9](#), [Figure 10](#)) showed a significant accumulation over the fault zones and were almost absent where the productive horizon becomes deeper, suggesting the oil-water contact. Oil analysis showed a very high vanadium content ([Figure 7](#)) supporting the micro seepage model due to hydrothermal activity ([Figure 9b](#)) exposed before ([Figure 4](#)). Vascular plants also expressed the microseep effect by having lower species diversity and richness values in the gas-affected zones. No reliable plant bio-indicator was found.

The Shannon Diversity Index showed higher values at different points over the butane trophic bacteria records, supporting the biogeochemical model ([Figure 6](#)). The same happened with the Specific Richness Index which reached the higher values outside the points with presence microbial anomalies ([Figure 7](#)). Note that the transect crossed floodplain areas of the Malargüe River, for which the soil had a high salt content. So it was decided to make a change in the recorded values of the halophyte species *Salicornia ambigua*, applying a logarithm to their frequency. This correction reduced its incidence so that the values of the Shannon Index (H) and therefore the presence of soil salinity did not mask the possible action of gas microleakages and its expression in the whole plant community ([Figure 11](#), [Figure 12](#), [Figure 13](#), [Figure 14](#)).

## Conclusions

The presence of butane-trophic bacteria and trace elements in the soil associated with faults suggests a case of hydrothermal activity (intrusive rock) related to reservoir microseepage may be acting as a carrier force for the gas and trace elements to reach the surface. The anomalous vanadium amounts found in soils confirm this theory, and it was first identified in the chemical analysis of an oil sample from a discovery well. The vascular plant community also responded to the microseeps by lowering its diversity and richness in the zones affected by the gas plume, probably because both bacteria and vascular plants use the same inorganic nutrients. Also, the high bacterial oxidizing activity localized in the

fault zones could have increased the CO<sub>2</sub> pressure of the soil and restricted the oxygen supply for roots metabolism. This local environmental pressure explains why some species can inhabit the gas-affected regions and some others cannot (see Larriestra et al., 2012).

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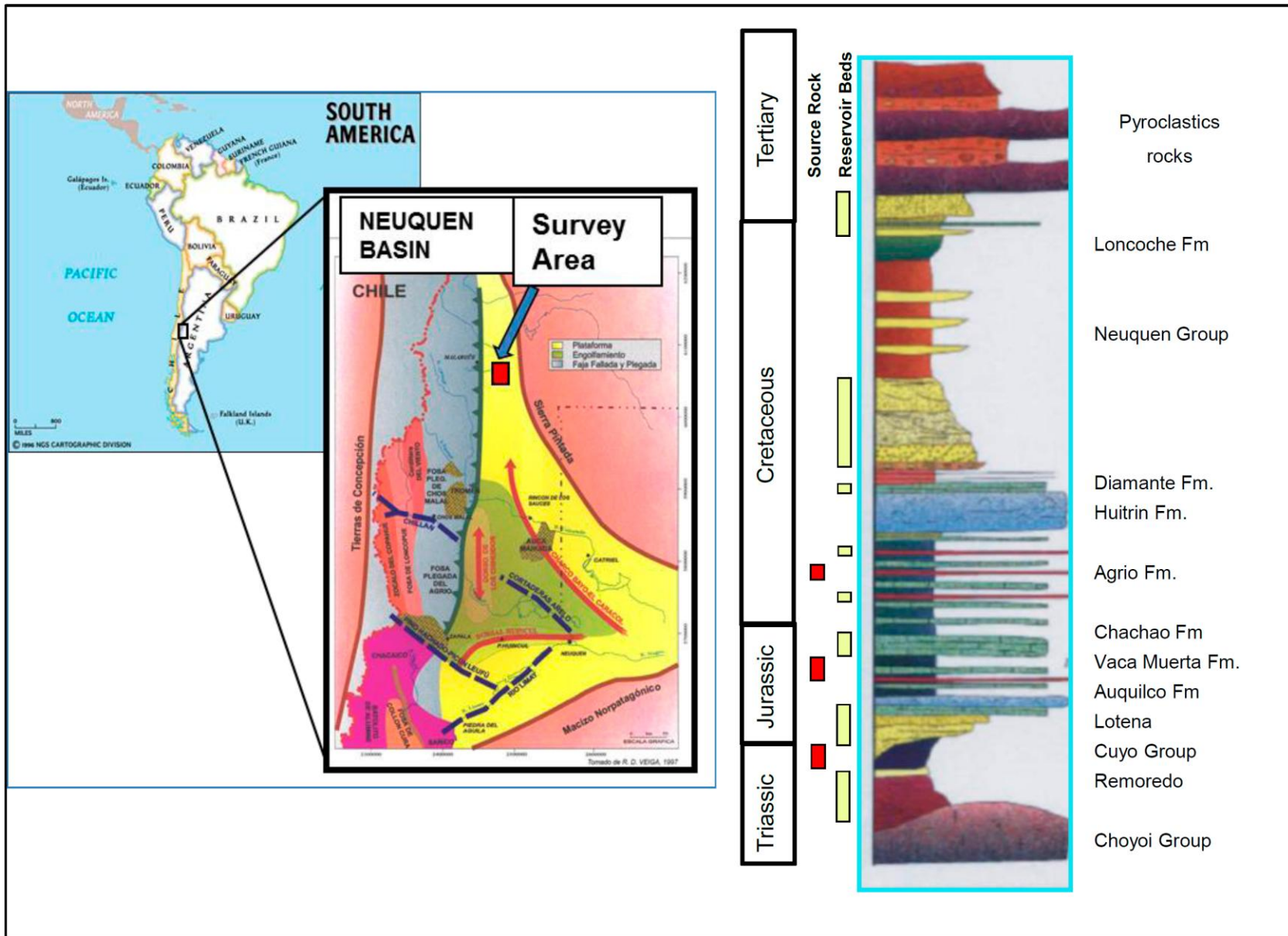


Figure 1. Location and geological setting.

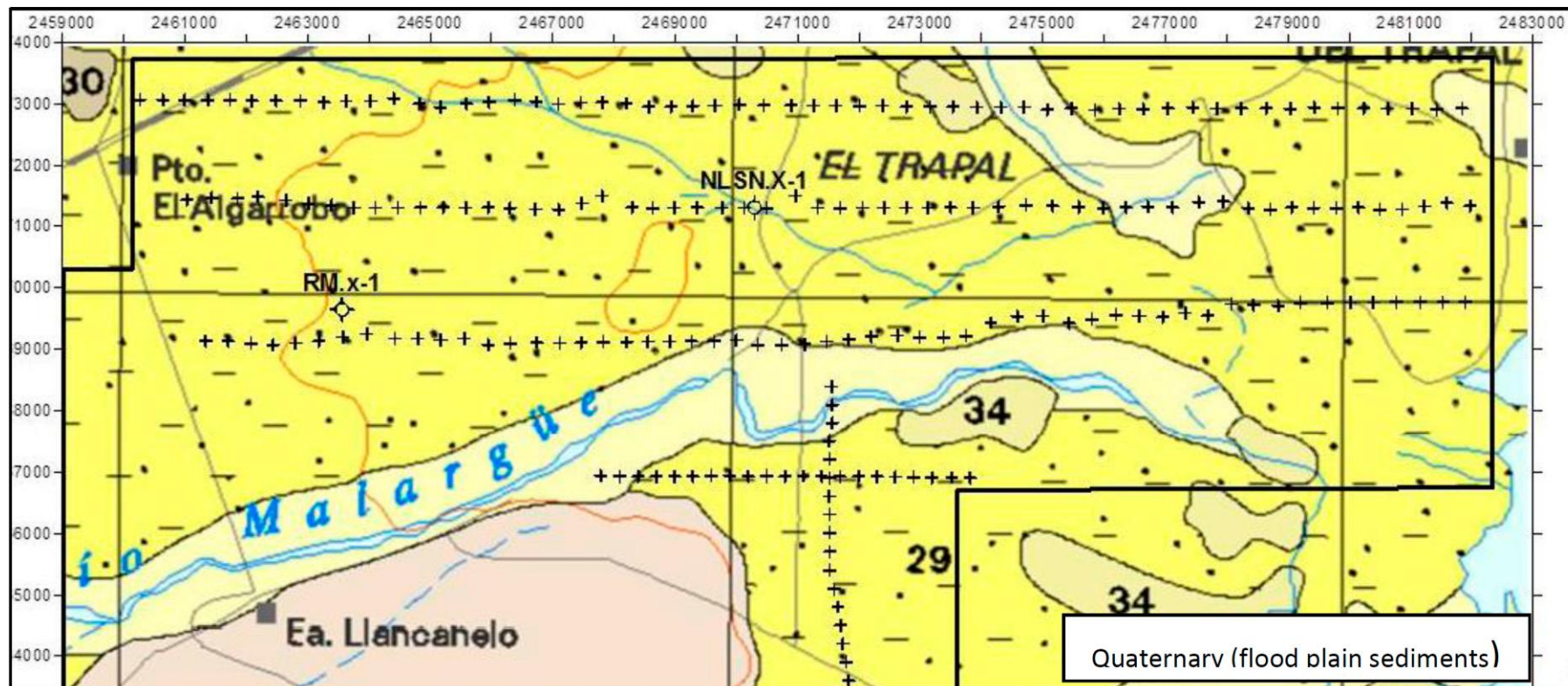


Figure 2. Geologic map (Nulo et al., 2005).



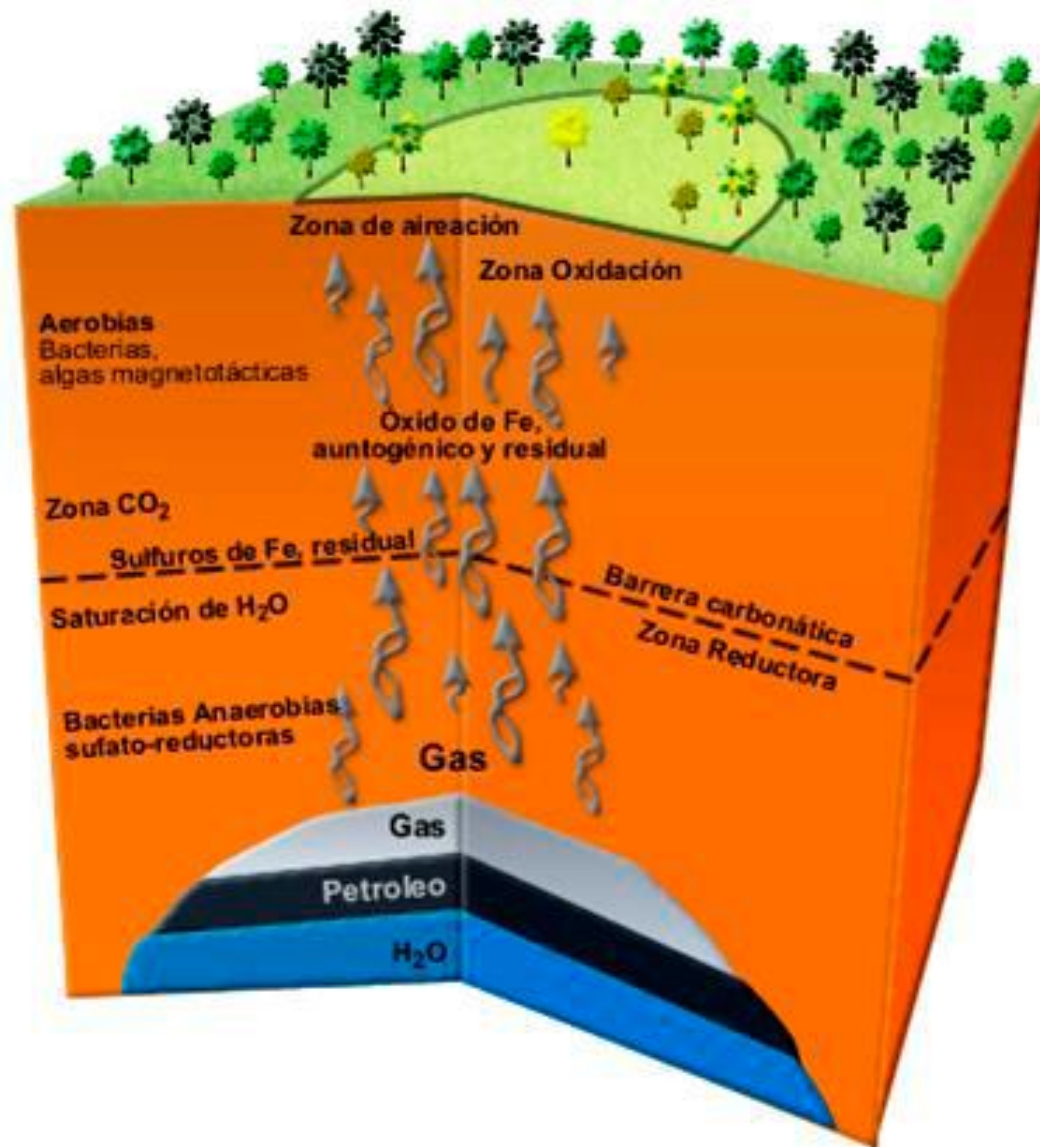


Figure 3. Biogeochemical model. Reservoir hydrocarbon gases rise up through the stratigraphic column creating a reduction chimney (grey arrows) that reaches the surface. This creates an anomalous zone with high soil pH values that has an impact on plant community structure and diversity.

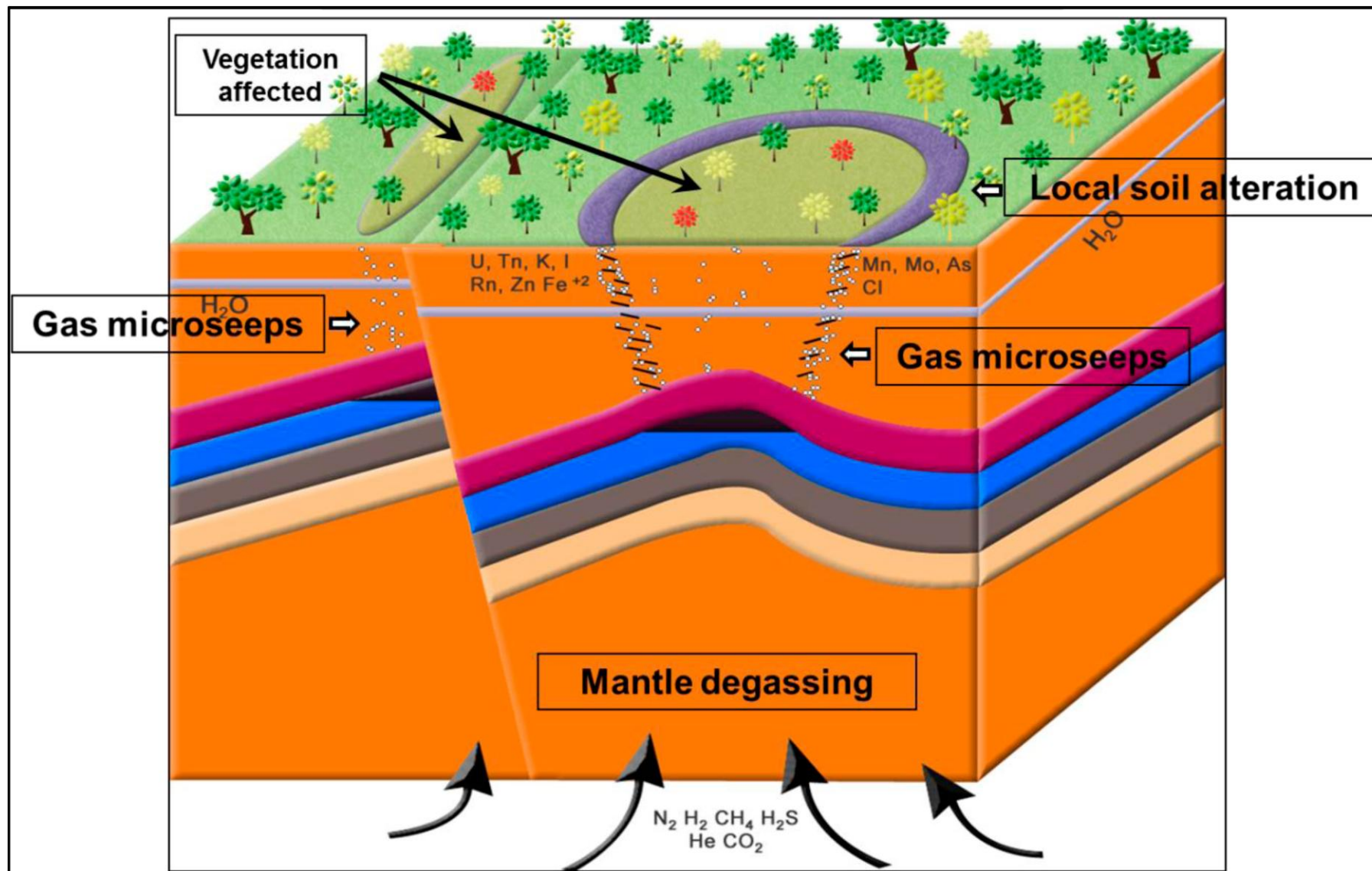


Figure 4. Inorganic geochemical model showing the carrier gases ( $H_2$ ,  $N_2$ ,  $CH_4$ ,  $He$ ,  $CO_2$ ,  $H_2S$ ) rising from the mantle and carrying elements related to reservoir rocks. This phenomenon creates an anomalous superficial deposit of characteristic trace elements and minerals that influences the local ecosystem.

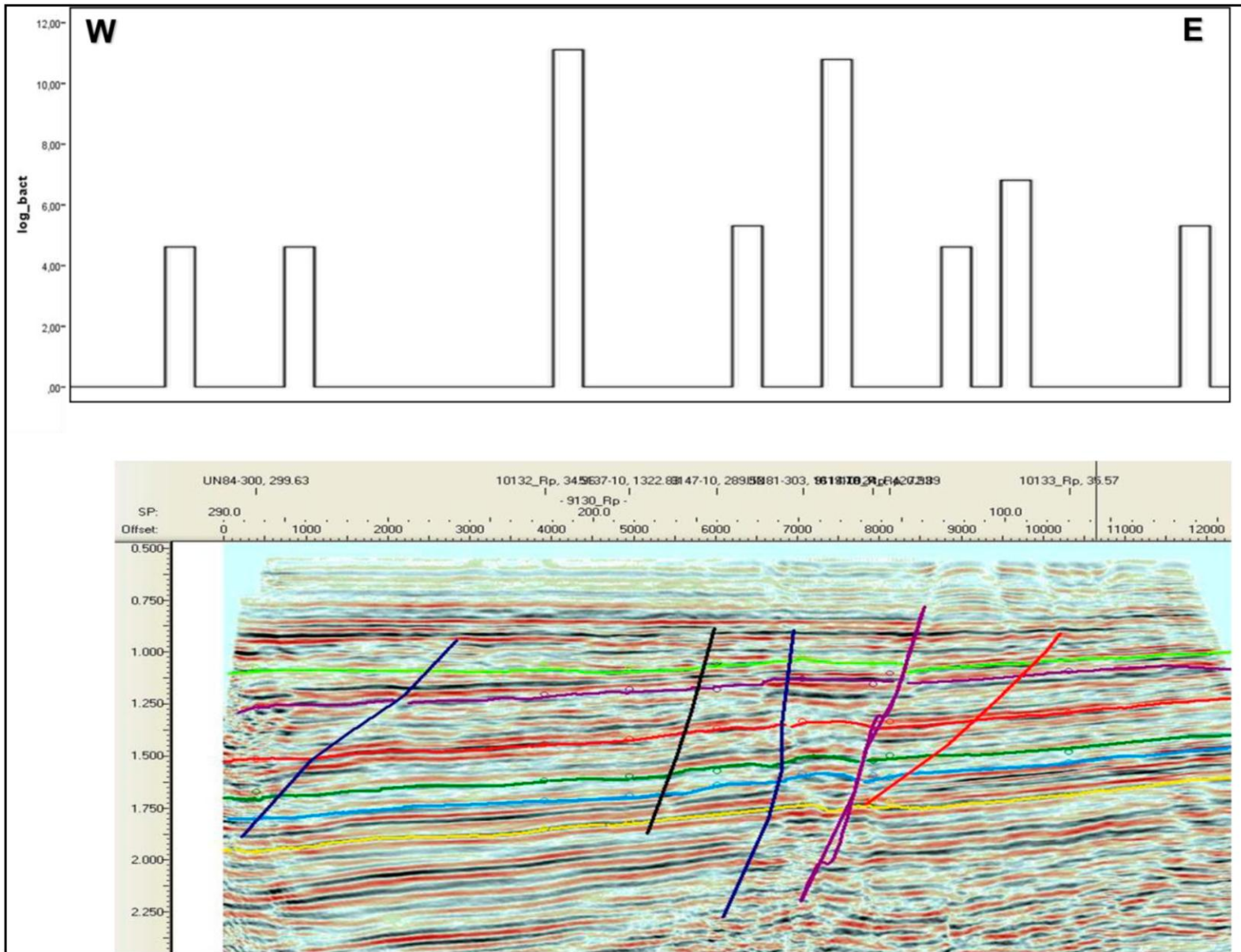


Figure 5. Natural logarithm of bacteria, showing its relationship with faults along the transect. The third great peak of bacteria UFC/g is out of this picture, in the eastern side.

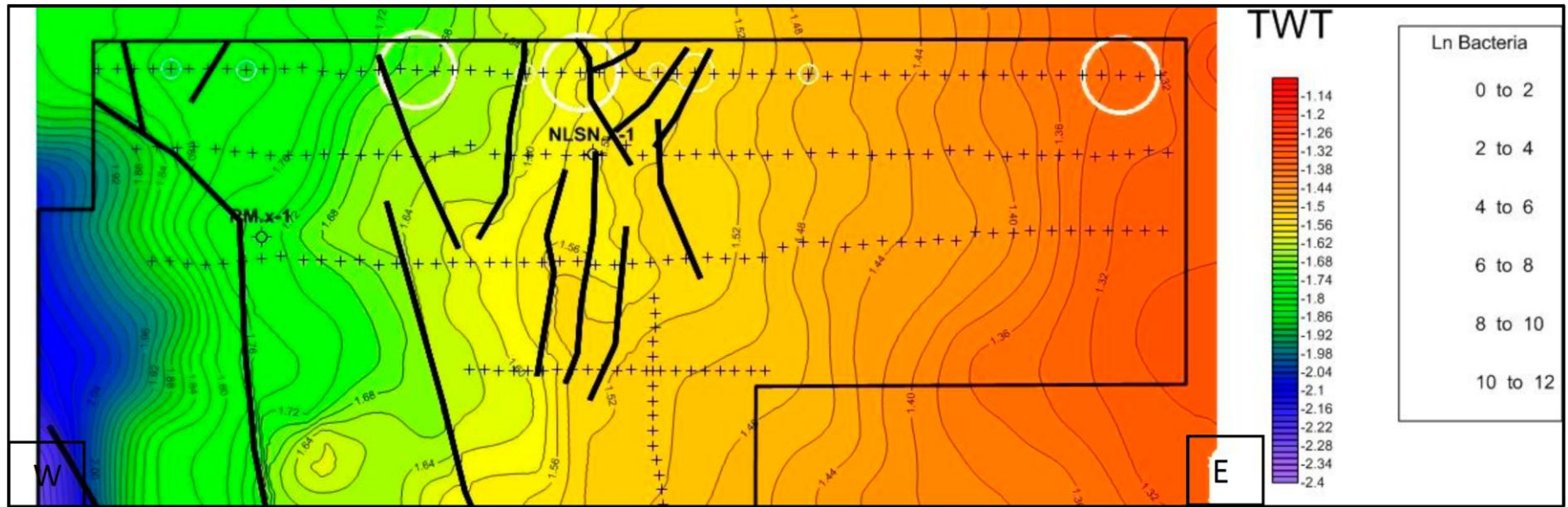


Figure 6. Time structure map of reservoir top and transect showing natural logarithm of bacteria counts, related to faults. The western side of the transect shows very weak bacterial activity and is close to the deepest part of the productive horizon.

| FRX        | Mo    | Zr    | Ni    | Fe    | V     | K       | S     |
|------------|-------|-------|-------|-------|-------|---------|-------|
| registro 1 | 23.16 | 13.21 | 50.33 | 103.5 | 482.8 | 967.09  | 26088 |
| registro 2 | 23.62 | 14.66 | 71.69 | 114.6 | 490.1 | 1084.44 | 25132 |

Figure 7. Assay of oil samples showing anomalous vanadium content.

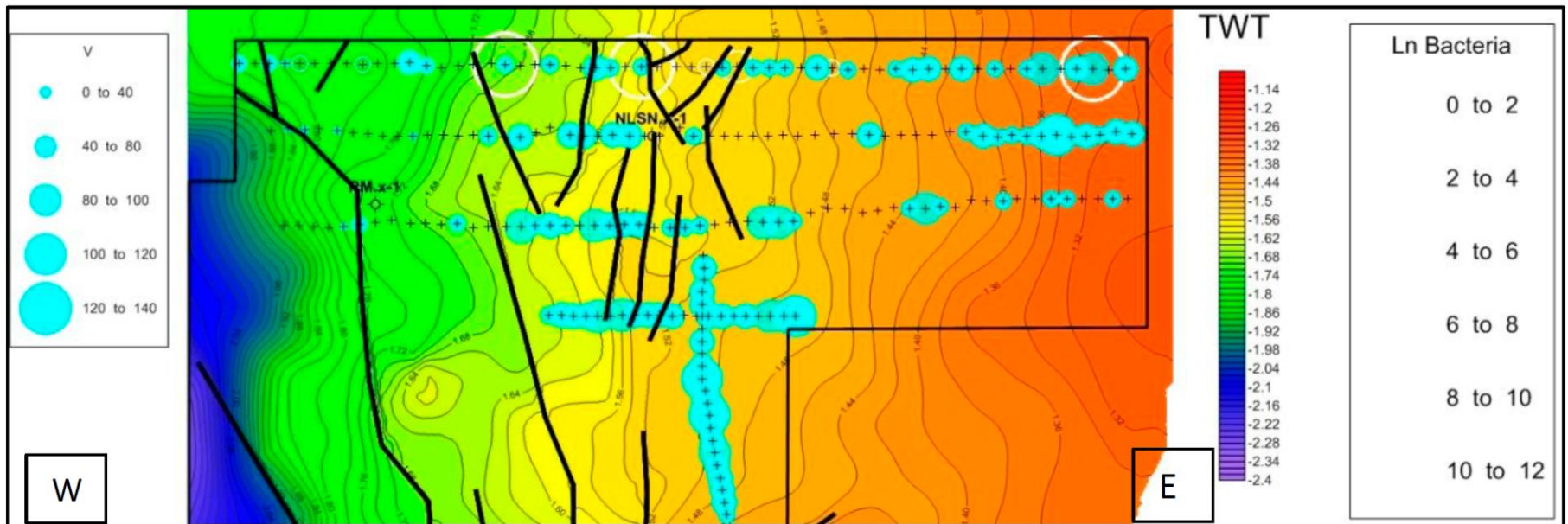


Figure 8. Time structure map of reservoir top with vanadium (ppm) bubble map (light blue) and bacteria peaks (white circles).

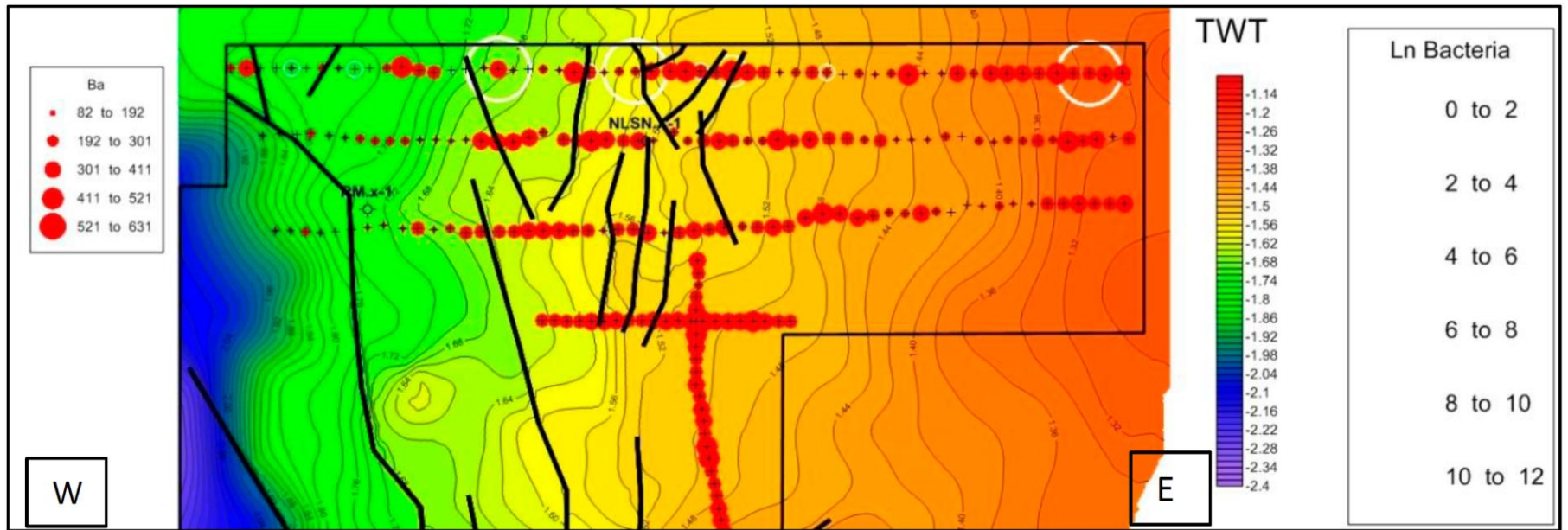


Figure 9a. Time structure map of reservoir top with barium (ppm) bubble map (red) and bacteria peaks (white circles).

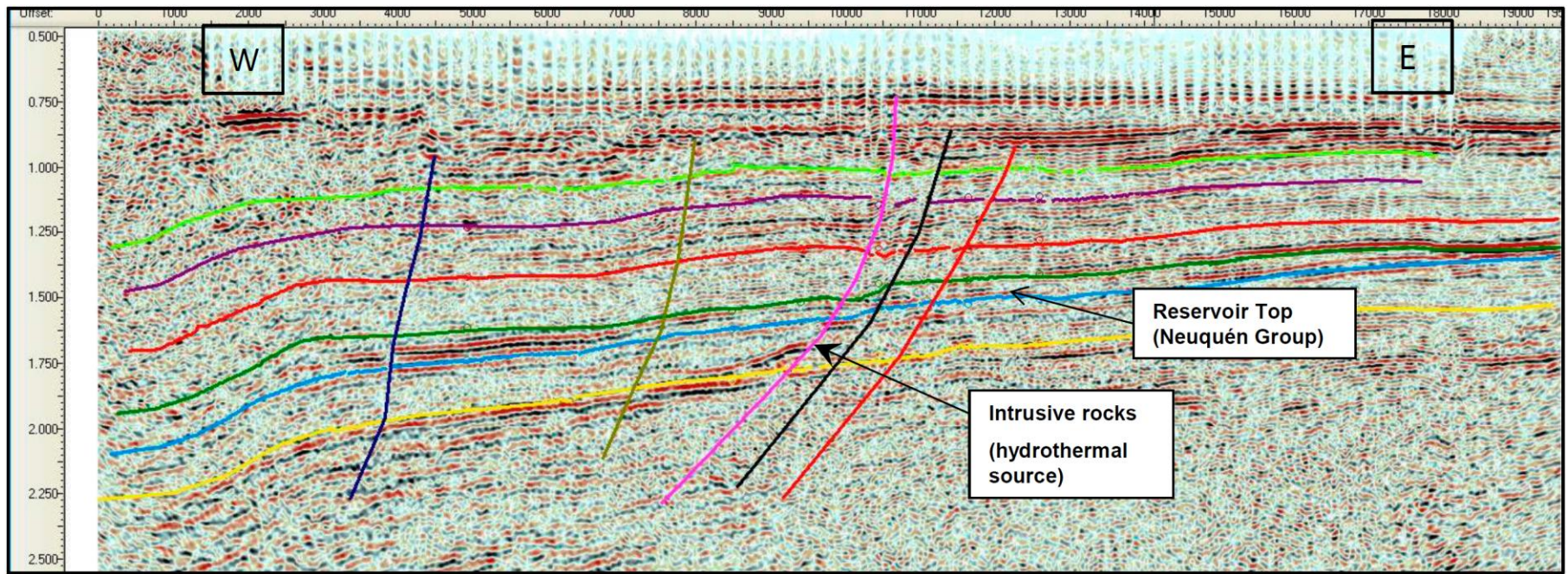


Figure 9b. Parallel seismic section showing evidence of hydrothermal activity due to basic intrusive rocks probably related to barium concentration in the soil.



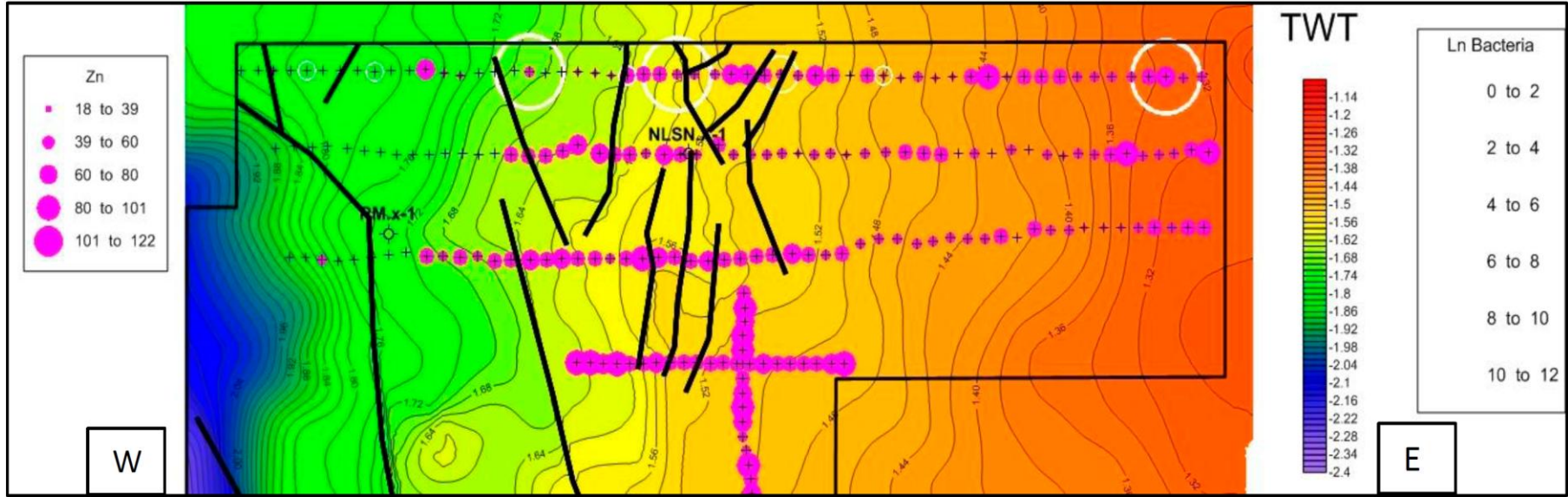


Figure 10. Time structure map of reservoir top with zinc (ppm) bubble map and bacteria peaks (white circles).



Figure 11. NDVI image on which the Shannon Diversity Index (H) magnitudes (orange bubbles) and bacterial values (black circles with values in CFU/g). NDVI image showing Malargüe River floodplains in shades of brown and plant cover in green. Note that diversity increases in non-anomalous areas.

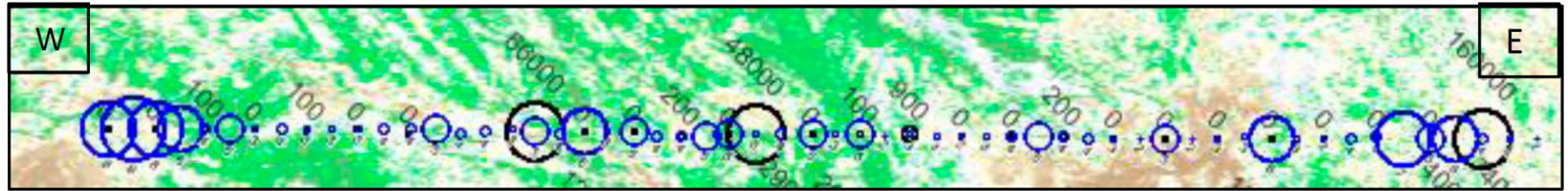


Figure 12. NDVI image showing Specific Richness (blue circles) and bacterial counts (black circles values of CFU/g). The total number of species present in the sample stations is not affected by gas and bacteria tends to be higher.

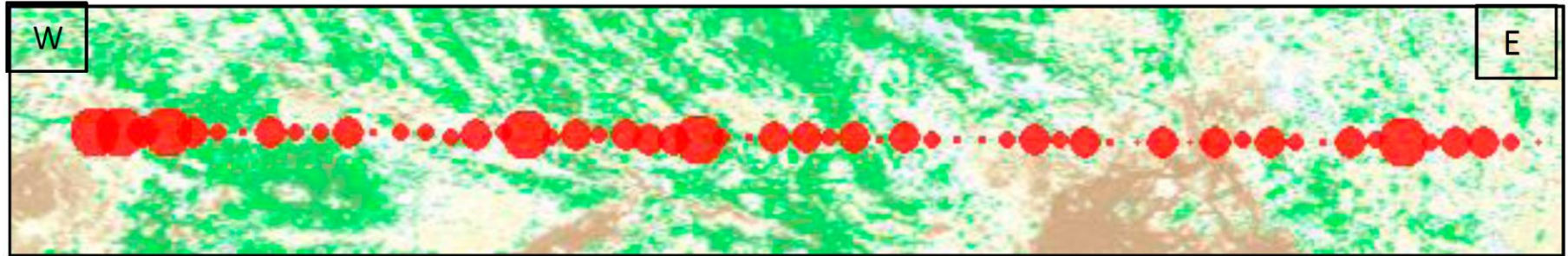


Figure 13. Shannon index (H) without applying  $\log_2$  values to the frequency of individuals of *Salicornia ambigua* (there appears to be a considerable diversity of values in the saline floodplains).

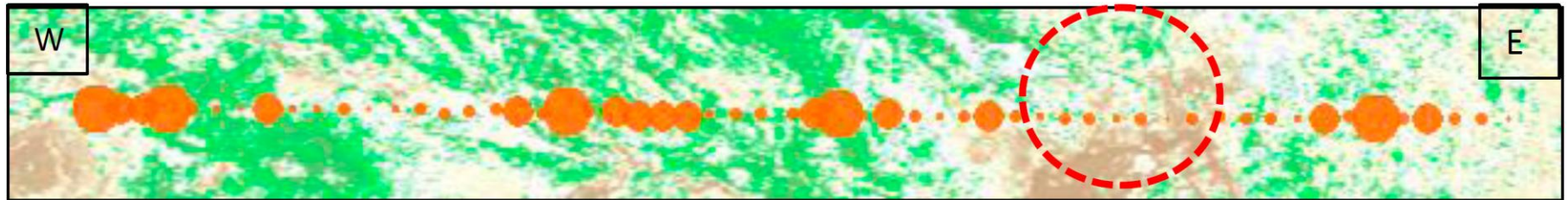


Figure 14. Shannon index (H) applying natural logarithm correction to the frequency of *Salicornia ambigua*. Note that the diversity index decreases in saline floodplain areas.