

# Toxicity Measurement of Sediments with Accepted Anomalous Deliberation in Heavy Metals by the Exploit of Bioassay

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## Abstract

The potential toxicity in riverbed sediments was assessed with a bioassay by means of the bioluminescent bacteria *Vibrio fischeri*. The elected area was characterized by the attendance of ultramafic rocks (peridotites), and the sediments had elevated values in Ni, Cr, and Co. For the toxicity bioassay with *Vibrio fischeri*, water-soluble forms were worn. The results indicated that mainly of the samples had a very low quantity of toxicity, with 14% of decrease in luminescence in relative to the organize; meanwhile 29% of the samples had a reasonable degree of toxicity with a decrease in luminescence among 15 and 23% in relation to the manage. The toxicity index interrelated considerably with the concentrations of Ni and Cr in the water extracts. This toxicity bioassay was proved to be a responsive and functional tool to detect potential toxicity in solutions, even with inconsistent concentrations in heavy metals of ordinary origin. . Ecological risk assessments can be worn to anticipate the likelihood of future belongings or approximation the likelihood that effects are caused by past opening to stressors.

**Keywords:** Toxicity Bioassay, *Vibrio fischeri*, Verde River, Luminescence, ANOVA.

## I. INTRODUCTION

An ecological risk measurement is the progression for evaluating how likely it is that the surroundings may be impacted as a result of contact to one or more environmental stressors such as chemicals, land change, sickness, enveloping species and climate alteration. Every day, people features questions about environmental concerns, many of them connected to plants, animals, ecosystems as a complete, and how we interrelate with them. These questions may be about potential risks such as impacts on the aesthetic value of a place due to physical alterations; belongings of pollution on endanger species, or the consequences of long-term discharge of contaminants to an environment. Ecological risk assessments can be worn to expect the likelihood of future belongings or estimate the likelihood that effects are caused by past introduction to stressors. Information from environmental risk assessments are then used by risk managers for follow-up such as communicating to concerned parties and the universal public, preventive activities related to the ecological stressor, limiting use of a given substance, or developing a monitoring plan to conclude if risks have been condensed or whether an ecosystem is improving.

Most bioassays practical to infected soils and sediments are based on the assessment of the toxic consequence of the solution extracted from the solid phase or by the solid phase itself over an existing creature. In this way, bacterial bioassays are frequently

used because they are speedy, cost successful, and reproducible. Principally, the bioassay using *Vibrio fischeri* relates the attendance of contaminants to the reticence in light production from these luminescent bacteria. This test is definite as responsive and has a high correlation with the response of other toxicity tests; in addition, it has been used in the toxicity measurement of soils infected by heavy metals.

Rivers distribute important metals in the ecology by mobilizing pollutants and thus dispersal the artificial area, with potential toxicity risk to aquatic organisms as well as to human health throughout the food sequence. Heavy metals can reach water ecosystems by anthropic behavior or by natural processes, and in such conditions; the contaminants can be circulated as water-soluble species, colloids, suspended forms, or sedimentary phases. Heavy metal contamination in aquatic ecosystems has established increased scientific concentration in the recent years because the contaminants tend to accrue and increasingly raise the toxicity risk to the living organisms. In this sense, many studies have established that heavy metal concentration in river bed sediments can be good indicators of contamination in hydrological systems.

Heavy metals can be bound to or occluded in amorphous materials, adsorbed on clay surfaces or

iron/manganese ox hydroxides, co precipitated in secondary minerals such as carbonates, sulphates, or oxides, complexes with organic matter, or included in the lattice of primary minerals such as silicates. The fractionation techniques of heavy metals in the river sediments have been worn by different authors to assess the mobility and bioavailability of pollutants in this media.

The Verde River basin is positioned in the Province of Malaga, and its catchment area receives many streams graceful over peridotites materials, characterized by high concentrations of Mn, Cr, Co, and Ni. In this sink lies La Concepcion 'on Reservoir, which contributes with more than 24% of the consumption water worn in the western Costa del Sol, one of the main traveler areas in Spain and in southern Europe. The abovementioned scenario prompted the assessment of the riverbed sediments of this area. In this learning, we analyse the concentration in the riverbed sediments of important metals, both total as well as water soluble forms, to characterize the potential mobility of these elements in the Verde River basin. The potential toxicity of heavy metals was calculated using bioassay of bioluminescent bacteria in order to assess the impending risk of contagion in the area.

## II. MATERIAL AND METHODS

Verde River is in the order of 36 km long, originating in the Sierra de Las Nieves Mountains (2000 m.a.s.l.) and sharply downward to 400 m to reach the Mediterranean Sea. This unexpected change in elevation in a short detachment involves many dissimilar slopes, with the steeper ones predominating (27%–57%). The litho logy is conquered by peridotites and meandering rocks and with carbonate and metamorphic rocks in lesser quantity. The catchment area is comprised of the main conduit of the Verde River and 11 tributaries, counting La Concepcion 'on reservoir, investment 44,515hm<sup>3</sup>/year.

Sediments of the Verde River and main tributaries were composed in the bottom part of each stream. At each example point, composite samples were taken by integration 250 g of sediments from each bend and center of a square 0.5m per side. Samples were taken from the river bed to 0–20 cm intensity. In the laboratory, samples were air dried out, and the fine fraction (<50 μm) of the sediments was worn to differentiate the main properties for the toxicity bioassay.

The total important metals were resolute by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) in a PE SCIEX ELAN-500A spectrophotometer.

The analyses were completed after acid digestion (HNO<sub>3</sub> + HF; ratio 2:3) at a high warmth and pressure in a Teflon-lined container. The spectrometer was prepared with quartz torch, nickel sampler, and skimmer cones, a cross-flow type pneumatic nebulizer, and a double-pass Scott-type spray compartment.

Instrumental drift was monitored by frequently running ordinary element solutions connecting samples. The water soluble forms were obtained from sediment-water remove in a ratio of 1: 5, and the heavy metals solubilized were also unwavering by ICP-MS. All ICP-MS standards were equipped from ICP single-element average solutions (Merck quality) after apposite dilution with 13% HNO<sub>3</sub>. For calibration, two sets of multielementare standards containing all the analyses of concentration at five concentrations were organized using rhodium as an internal regular.

The toxicity bioassay was made with the water extract of the sediment. Prior to the assay, pH was measured potential metrically in a 1: 5 soil: water deferment in a CRISON 501 instrument, and electric conductivity (EC) was considered at 25°C in a CRISON 522 apparatus. The toxicity bioassay was completed with bacterium (*Vibrio fischeri*), which diminishes its bioluminescence capability in the attendance of toxic elements. The freeze-dried luminescent bacteria (NRLLB-11177) and the reconstitution explanation were complete by AZUR Environmental. The test was performed in a Microtox 500 analyzer from Microbics Corporation, according to a modification of Microtox Basic Test for Aqueous Extracts Protocol, in which the water-sediment extracts and a control sample (distilled water) were used, with three replicates per sample. The luminescence was measured before the mixture with the extracts (0 min). The embarrassment of bioluminescence was calculated at 7 (Inh7) and 17 minutes (Inh17) after the assortment with the extracts of the samples. Afterwards, these measurements were used to calculate two Toxicity Indexes:

- Normalized inhibition of luminescence at 7 min (I7), calculated by:

$$I7 = - (\text{Inh7}_{\text{sample}} - \text{Inh7}_{\text{control}}) / 100 - \text{Inh7}_{\text{control}} \quad (1)$$

Where Inh7 sample is the percentage of luminescence reduction in the samples at 7 min, and Inh5control is percentage of luminescence reduction of control at 7min.

- Normalized inhibition at 17min (I17), calculated by:

$$I17 = - (\text{Inh17}_{\text{sample}} - \text{Inh17}_{\text{control}}) / 100 - \text{Inh17}_{\text{control}} \quad (2)$$

Where  $Inh_{17sample}$  is the percentage of reduction of the sample at 17 min, and  $Inh_{17control}$  is the percentage of reduction of control at 17 min;

The values of  $I_5$  and  $I_{15}$  can range from  $-1$  (maximum toxicity) to  $>0$ , and the subsequent classes can be recognized: (a) 0 to  $-0.25$  low, (b)  $-0.25$  to  $-0.5$  moderate, (c)  $-0.5$  to  $-0.75$  high, and (d)  $-0.75$  to  $-1$  very high toxicity. Values  $>0$  would indicate stimulation of the luminescence (hormesis).

### III. RESULTS AND DISCUSSION

The entire concentrations of heavy metals in the sediments (Table 1) designate that the peridotites materials have very elevated concentrations in Cr, Ni, Mn, and Co while in the supplementary materials (carbonate and metamorphic rocks) the standards of these elements are low, and the concentrations in Zn and As are advanced than in the peridotites area; the differences in Pb are not statistically momentous connecting the two types of materials. Consequently, the total concentrations in heavy metals in the sediments of the Verde River are honestly related to the dissimilar parent materials nearby in the area.

**Table 1: Total Heavy-Metal Concentrations (Mg Kg<sup>-1</sup>) in Sediments From Peridotites Materials and From Other Materials in the Verde River Basin.**

| Elements | Peridotites      | Other materials |
|----------|------------------|-----------------|
| Mn       | 1244.95 ± 81.92  | 708.07 ± 165.37 |
| Cr       | 1040.79 ± 131.15 | 236.00 ± 125.62 |
| Co       | 114.86 ± 14.21   | 34.00 ± 13.49   |
| Ni       | 1833.26 ± 232.46 | 372.78 ± 273.77 |
| Cu       | 23.93 ± 1.45     | 32.50 ± 4.09    |
| Zn       | 69.40 ± 6.10     | 166.64 ± 67.25  |
| As       | 4.94 ± 0.80      | 26.41 ± 13.30   |
| Pb       | 19.43 ± 4.09     | 21.75 ± 6.97    |

**Table 2: Water-Soluble Heavy-Metal Concentrations (Mg Kg<sup>-1</sup>) In Sediments From Peridotites and from other Materials in the Verde River Basin.**

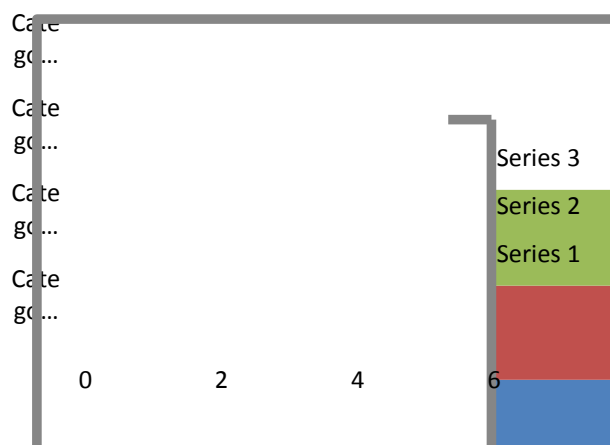
| Elements | Peridotites     | Other materials |
|----------|-----------------|-----------------|
| Mn       | 0.497 ± 0.245   | 0.280 ± 0.151   |
| Cr       | 0.013 ± 0.004   | 0.002 ± 0.001   |
| Co       | 0.015 ± 0.006   | 0.003 ± 0.001   |
| Ni       | 0.153 ± 0.048   | 0.008 ± 0.002   |
| Cu       | 0.009 ± 0.002   | 0.008 ± 0.002   |
| Zn       | 0.019 ± 0.006   | 0.011 ± 0.006   |
| As       | 0.005 ± 0.001   | 0.004 ± 0.002   |
| Pb       | 0.0004 ± 0.0003 | 0.0004 ± 0.0003 |

The highest heavy-metal concentrations were for Ni and Cr, with greatest values of 2552 mg kg<sup>-1</sup> and 1514 mg kg<sup>-1</sup>, correspondingly. According to the geochemical background of the outline elements in

soils of Andalusia, the sediments of the revise area have anomalous principles only for Ni, Cr, and Co in the peridotites materials, with concentrations exceeding, respectively, 36-, 10-, and 2-fold the reference values for the region. The concentrations of the other elements were within the normal range in all cases.

For the assessment of the potential toxicity of the samples, water extracts of the sediments were obtained to make the toxicity bioassay using luminescent bacteria. The main variables affecting the measurement in the bioassay were pH and electric conductivity (EC); these properties should be resolute to evaluate their influence in the test results. The water extract of the samples had a pH value of  $8.03 \pm 0.13$ , and the mean value of EC was  $1.36 \pm 0.12$ . These values are within the recommended range for this toxicity bioassay. The concentration of soluble heavy metals in the water extracts are presented in Table 2. The sediments coming from the peridotites area had significantly higher concentrations in soluble Ni, Cr, and Co than the sediments coming from other materials. The other elements analyzed had no significant differences in their soluble concentration between the different materials considered.

According to the toxicity bioassay with *Vibrio fischeri*, most samples showed a decrease in the luminescence in relation to the initial value (Figure 1). Because this bacterium is from a marine environment, the control samples (distilled water) had also a luminescence decrease of among 27 and



**Figure 1: Luminescence Inhibition (%) of the Water Extract in the Sediment Analyzed (C = control sample).**

34%. The water remove of the samples showed a lessening at 7min ( $Inh_{7sample}$ ) and 17 min ( $Inh_{17sample}$ ) below 50% in relative to the initial value in all cases even though these values were normalized to compute the embarrassment in relation to the

organize. The lower reticence of luminescence was found in the sediments belonging to the non peridotites area (samples 5, 6, and 7) and in sample 12, which established a mixture of deposit both from the peridotites materials as well as from the metamorphic carbonate region.

In the case of the sediments coming from the non-peridotites area or from a mixture of different parent materials (samples 5, 6, 7, and 12), the toxicity index had values higher than zero, indicating the occurrence of hormesis phenomena related to the stimulation of the bacterial activity. Only one sample

(4) had values of the toxicity index close to  $-0.25$  (representing a 27% reduction in luminescence with respect to the control), indicating a moderate degree of toxicity. The ANOVA of the toxicity index indicated that samples 1, 4, and 11 (located in the lower part of the peridotites area) significantly differed in relation to the other samples analyzed (Table 3), with a toxicity index ranging from  $-0.13$  to  $-0.21$ ; therefore, these three samples had a luminescence reduction of more than 10% but less than 27%, which could be related to the heavy-metal concentrations in the water extracts used in the bioassay.

**Table 3: Toxicity Index of the Water Extract at 5 min (I5) and 15 min (I15). (M: mean; SD: standard deviation; a, b: Significant Differences ( $P < .05$ ) in Tukey test).**

| Sample     | 1  | 2     | 3     | 4     | 5     | 6    | 7    | 8     | 9     | 10   | 11    | 12    |       |
|------------|----|-------|-------|-------|-------|------|------|-------|-------|------|-------|-------|-------|
| <b>I7</b>  | M  | -0.13 | -0.01 | -0.07 | -0.21 | 0.10 | 0.05 | 0.04b | -0.03 | 0.01 | -0.03 | -0.14 | 0.08b |
|            |    | a     | b     | b     | a     | b    | b    |       | b     | b    | b     | a     |       |
|            | SD | 0.02  | 0.07  | 0.08  | 0.05  | 0.10 | 0.03 | 0.02  | 0.01  | 0.08 | 0.03  | 0.03  | 0.10  |
| <b>I17</b> | M  | -0.15 | -0.02 | -0.09 | -0.19 | 0.08 | 0.08 | -0.03 | -0.06 | 0.00 | -0.04 | -0.15 | -0.01 |
|            |    | a     | b     | b     | a     | b    | b    | b     | b     | b    | b     | a     | b     |
|            | SD | 0.02  | 0.09  | 0.05  | 0.03  | 0.09 | 0.05 | 0.02  | 0.04  | 0.08 | 0.05  | 0.01  | 0.05  |

#### IV. CONCLUSIONS

The study area is dominated by peridotites materials, and the riverbed sediments in the basin have high concentrations of Ni, Mn, Cr, and Co. The soluble forms were from the water extract of the sediments of the Main River and tributaries in the basin. The toxicity bioassay with *Vibrio fischeri* used the water extract of these sediments to assess the bioluminescence decrease in these bacteria. The toxicity degree was very low in 76% of the samples, with values of luminescence reduction below 13% in relation to the control. A moderate-to-low degree of toxicity was found in 27% of the samples, with a luminescence reduction between 15 and 23% in relation to the control. The correlation coefficient indicated a negative and considerable relation connecting the toxicity index and the concentrations in Ni and Cr in the water extracts of the sediments. This toxicity bioassay was proved to be a responsive and useful tool for detecting the prospective toxicity of solutions, even in samples with inconsistent concentrations in heavy metals of accepted derivation.

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