Supplementary material

**An integrative approach to assess the environmental impacts of gold mining contamination in the Amazon.**

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Table S1. Geographic coordinates and description of collection sites. Depth at the center of the main river channel and width at the sampling location are reported.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sites | Coordinates | Elevation (m) | River | | | Local description |
| Name | Depth (m) | Width (m) |
| P1 | 1°06′27″S - 77°51′35″O | 603.8 | Río Chumbiyacu | 0.26 | 2.4 | P1 is in the Moretecocha mining area, within a forest area. The stream receives the wastewater from the waste pools. The mining machines were in operation during the sampling. |
| P2 | 1°08′17″S - 77°52′44″O | 577.9 | Río Chucapi | 0.12 | 6 | P2 is in a river that drains to an abandoned mining area. There was presence of secondary vegetation on the banks of the river. |
| P3 | 1°08′11″S - 77°52′41″O | 586.5 | Río Chucapi | 0.08 | 0.6 | P3 is located within an active mining area. The stream receives the wastewater from the waste pools. The mining machines were in operation during the sampling. |
| P4 | 1°06′55″S - 77°52′05″O | 580.0 | Río Chumbiyacu | 0.34 | 4 | P4 is in an area highly affected by the opening of a road. Construction machines were crossing the river and moving sediments from river margins and riverbed. |
| P5 | 1°06′33″S - 77°50′10″O | 502.4 | Río Chumbiyacu | 0.33 | 6 | P5 is in an abandoned mining area. Presence of secondary vegetation. The river receives water from mining areas. |
| P6 | 1°06′7″S - 77°49′00″O | 465.2 | Río Chumbiyacu | 0.19 | 14 | P6 is located near Chumbiyacu River bridge, downstream from point P5. |
| P7 | 0°59′13″S - 77°31′01″O | 360.9 | Río Huambuno | 0.45 | 0.5 | P7 is located about 50 m upstream of an active mining area. |
| P8 | 0°58′20″S - 77°29′51″O | 355.8 | Río Huambuno | 0.57 | 15 | P8 is situated near Huambuno River bridge, downstream from point P7. |
| P9 | 1°02′09″S - 77°36′43″O | 440.2 | Río Tuyano | 0.17 | 0.15 | P9 is located within an active mining zone. The mining machines were in operation during the sampling. The riverbed was totally modified for the benefit of filling waste pools and washing alluvial sediments. |
| P10 | 1°03′01″S - 77°48′42″O | 441.6 | Quebrada Yutzupino | 0.02 | 0.63 | P10 is located about 60 m from the Portoviejo highway, close to Puerto Napo. Downstream from a mining area. |
| P11 | 1°03′00″S - 77°48′42″O | 440.4 | Río Yutzupino | 0.26 | 0.04 | P11 is located about 40 m from the Portoviejo highway, close to Puerto Napo. Downstream from a mining area. |

**Integrative index based on Multiple Lines of Evidence**

For the LOE physicochemical and the LOE metals exposure assessment, two equal methods of evaluation were used to assign values into the integrated index (Table S2). First, we select only the physicochemical parameters and metal species above TULSMA (MAE, 2015) permissible limits. Then, we determine classes of values depending on how much these parameters exceed permissible limits on a 50 times-base, i.e., if values were 50 times lower than permissible limits, they received the highest score (low environment impact); if values were 50 times or more above the limits, scores were lower (high environmental impact). Secondly, we established a measure based on the total number of physicochemical parameters and metal species that exceed the permissible limits for each site. This measure is complementary to the first evaluation method because it is independent of the concentration or value of each parameter. In this case, if less than 20% of the physicochemical parameters and metals exceed the permissible limit, the higher score is assigned; if more than 80% of the physico-chemical parameters and metals species exceed the permissible limit, the lower score is assigned to the site.

For the LOE phytotoxicity of *L. sativa*, whenever growth enhanced or inhibition differ up to 20% from control, the higher score of 5 was assigned (low environmental impact); whenever growth inhibition differs 80% from control, the lower score was assigned (high environmental impact). For the mortality of *D. magna*, higher scores were assigned when neonates survival rate was above 80%, and lower scores were assigned when neonates survival rates were less than 20%. For the LOE AAMBI, the higher score was assigned to AAMBI values classified as “excellent (>121) and the lowest score was assigned when AAMBI values were classified as “bad” (<35).

Table S2. Parameters and the respective scores used to calculate the integrated index that includes the four LOE of freshwater parameters (Physico-chemical, Metal concentrations, Macroinvertebrates and Toxicity) assessed in the study area.

|  |  |  |
| --- | --- | --- |
| Ranking categories | | Score |
| Physico - chemical concentrations | 1- Physico-chemical values exceed limit permissible |  |
| Values are less than 50 times low of the limit permissible value | 5 |
| Values are between 50 times low of the limit permissible value until the limit permissible value | 3.6 - 4.9 |
| Values are from limit permissible value until 50 times upper of the limit permissible value | 1.8 - 3.5 |
| Values are between 50 to 100 times upper the limit permissible value | 0.1 - 1.7 |
| Values are more than 100 times upper of the limit permissible value | 0 |
| 2- Number of Physico-chemical parameters exceed limit permissible |  |
| Of the total amount of parameters, less than 19% of parameters exceed limit permissible | 4.1 - 5 |
| Of the total amount of parameters, between 39% to 20% parameters exceed limit permissible | 3.1 -4.0 |
| Of the total amount of parameters, between 59% to 40% parameters exceed limit permissible | 2.1 -3.0 |
| Of the total amount of parameters, between 79% to 60% parameters exceed limit permissible | 1.1 - 2.0 |
| Of the total amount of parameters, more than 80% of parameters exceed limit permissible | 0 - 1.0 |
| Metal concentrations | 3- Metals values exceed limit permissible |  |
| Values are less than 50 times low of the limit permissible value | 5 |
| Values are between 50 times low of the limit permissible value until the limit permissible value | 3.6 - 4.9 |
| Values are from limit permissible value until 50 times upper of the limit permissible value | 1.8 - 3.5 |
| Values are between 50 to 100 times upper the limit permissible value | 0.1 - 1.7 |
| Values are more than 100 times upper of the limit permissible value | 0 |
| 4- Number of Metals exceeding permissible limits |  |
| Of the total amount of parameters, less than 19% of parameters exceed limit permissible | 4.1 - 5 |
| Of the total amount of parameters, between 39% to 20% parameters exceed limit permissible | 3.1 -4.0 |
| Of the total amount of parameters, between 59% to 40% parameters exceed limit permissible | 2.1 -3.0 |
| Of the total amount of parameters, between 79% to 60% parameters exceed limit permissible | 1.1 - 2.0 |
| Of the total amount of parameters, more than 80% of parameters exceed limit permissible | 0 - 1.0 |
| Toxicity bioassay | 5 - Daphnia Magna |  |
| Neonates survives more than 80% | 4.1 - 5 |
| Neonates survives between 60% and 79% | 3.1 -4.0 |
| Neonates survives between 40% and 59% | 2.1 -3.0 |
| Neonates survives between 20% and 39% | 1.1 - 2.0 |
| Neonates survives less than 19% | 0 - 1.0 |
| 6- Phytotoxicity (*Lactuca sativa*) |  |
| Growth enhanced or inhibition until 20% from control | 4.1 - 5 |
| Growth enhanced or inhibition 21% to 40% from control | 3.1 -4.0 |
| Growth enhanced or inhibition 41% to 60% from control | 2.1 -3.0 |
| Growth enhanced upper to 60% from control and inhibition 41% to 60% from control | 1.1 - 2.0 |
| Growth inhibition upper 80% from control | 0 - 1.0 |
| Macroinvertebrates | 7 - AAMBI | |
| Excellent (value <121) | 5 |
| Very Good (range value 90 - 120) | 3.6 - 4.9 |
| God (range value 50 - 89) | 2.0 - 3.5 |
| Regular (range value 36 - 49) | 1.4 - 1.9 |
| Bad (<35) | 0 - 1.3 |