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Long-range effect of cyanide on mercury methylation in a gold mining area in southern Ecuador

Jean Remy Davée Guimaraes ^{a,*}, Oscar Betancourt ^b, Marcio Rodrigues Miranda ^a, Ramiro Barriga ^c, Edwin Cueva ^b, Sebastián Betancourt ^b

^a Laboratório de Traçadores, IBCCF/UFRJ, CCS, Bloco G, Av. Carlos Chagas Filho s/n, CEP 21941-902 Rio de Janeiro, RJ, Brazil

^b Health, Environment and Development Foundation, FUNSAD, Ecuador

^c National Polytechnic School, Ecuador

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ABSTRACT

Small-scale gold mining in Portovelo–Zaruma, Southern Ecuador, performed by mercury amalgamation and cyanidation, yields 9–10 t of gold/annum, resulting in annual releases of around 0.65 t of inorganic mercury and 6000 t of sodium cyanide in the local river system. The release of sediments, cyanide, mercury, and other metals present in the ore such as lead, manganese and arsenic significantly reduces biodiversity downstream the processing plants and enriches metals in bottom sediments and biota. However, methylmercury concentrations in sediments downstream the mining area were recently found to be one order of magnitude lower than upstream or in small tributaries. In this study we investigated cyanide, bacterial activity in water and sediment and mercury methylation potentials in sediments along the Puyango river watershed, measured respectively by in-situ spectrophotometry and incubation with ³H-leucine and ²⁰³Hg²⁺.

Free cyanide was undetectable (<1 µg·L⁻¹) upstream mining activities, reached 280 µg·L⁻¹ a few km downstream the processing plants area and was still detectable about 100 km downstream. At stations with detectable free cyanide in unfiltered water, 50% of it was dissolved and 50% associated to suspended particles. Bacterial activity and mercury methylation in sediment showed a similar spatial pattern, inverse to the one found for free cyanide in water, i.e. with significant values in pristine upstream sampling points (respectively 6.4 to 22 µgC·mg wet weight⁻¹·h⁻¹ and 1.2 to 19% of total ²⁰³Hg·g dry weight⁻¹·day⁻¹) and undetectable downstream the processing plants, returning to upstream values only in the most distant downstream stations. The data suggest that free cyanide oxidation was slower than would be expected from the high water turbulence, resulting in a long-range inhibition of bacterial activity and hence mercury methylation. The important mercury fluxes resultant from mining activities raise concerns about its bi-methylation in coastal areas where many mangrove areas have been converted to shrimp farming.

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1. Introduction

The Puyango River basin is situated in South Western Ecuador, near the border with Peru and has been a site of gold and silver mining since pre-colonial times, leading to the present name of the province, El Oro. In the Portovelo–Zaruma region, upper Puyango basin, larger scale mining by foreign companies produced 1000 t of Au, 500 t of Ag and 1500 t of Cu during the first half of the 20th century (Astudillo, 2007). In the 1980s, an economic crisis combined with a sharp increase in gold prices led to the invasion of abandoned mines, resulting in a boom of small-scale mining. Today, over 110 small riverside processing plants operate in the region, extracting approximately 9–10 t of gold per annum by a combination of amalgamation with Hg and cyanide leaching from ore coming from over 400 mines. This combination of extraction processes

is not exclusive to Ecuador and is used in other developing countries for the same reason, namely the low gold recovery when using Hg amalgamation alone. Velasquez-Lopez et al. (2010, 2011) described in close detail the extraction processes used by the miners from Portovelo–Zaruma and, more important, the inventory of the resultant Hg and cyanide emissions in each step of the process. Besides gold, the sulfur-rich polymetallic ore they process contains high concentrations of Mn, Cd, Pb, As, Cu and Zn and inadequate management of mine tailings results in the discharge of toxic cyanide effluents and of sediments highly enriched with Hg and other metals, and the signature of mining activity in sediments and suspended particles is detectable far downstream (Tarras-Wahlberg et al., 2001; Betancourt et al., 2005). In the 2000 dry season Hg in suspended sediments 100 km downstream the processing plants was ~400 ng·g⁻¹·dry weight and it increased by one order of magnitude in the wet season, while for lead the concentrations were 250 and 2600 µg·g⁻¹ in the dry and wet season respectively.

* Corresponding author. Tel.: +55 21 25626651.

E-mail address: jeanrdg@biof.ufrj.br (J.R.D. Guimaraes).

The cyanidation plants operate in a hilly region and due to the scarcity of flat areas the mining facilities and cyanidation plants are built directly on the river banks and as a consequence cyanidation plants respond for $\pm 80\%$ of the sediment slurry laden by mining activities to the river (Prodeminca, 1998). In May 2011, over 110 cyanidation plants were operating in the region, releasing estimated 880,000 t/a of tailings into the river (Veiga, 2011). The Puyango river flows into the Tumbes River that reaches the Pacific Ocean, transporting mining-derived metals to the coastal areas, affecting mangroves and large shrimp farms. Studies in the 70s and 80s have estimated that about 289 kg of Hg was annually discharged into the Calera River in Portovelo–Zaruma (Prodeminca, 1998) and recently Velasquez-Lopez et al. (2010) estimated an annual discharge of 430 kg of Hg.

Betancourt et al. (2005) studied the impact of mining on environment and human health along the Puyango river basin, documenting the occupational exposure to Pb and Hg in the upper basin, where the intake of fish and river water are low, and the high exposure to lead in the semi-arid lower basin, where the population relies heavily on the river for fish and water supply. They found very low suspended particle loads upstream the mining area, ranging from 1.6 to $3.0 \text{ mg} \cdot \text{L}^{-1}$ depending on the season, while near the processing plants, levels ranged $132\text{--}328 \text{ mg} \cdot \text{L}^{-1}$, and similar profiles were found for metals in river sediments, with maximum mercury concentrations of $0.061 \mu\text{g} \cdot \text{g}^{-1}$ upstream mining activities and $0.730 \mu\text{g} \cdot \text{g}^{-1}$ downstream.

However, Roulet et al., 2006 found that methylmercury was at least one order of magnitude lower in sediments downstream the mining area when compared to levels upstream or in small tributaries unaffected by mining activities, both as absolute concentrations or as % of total mercury and raised the hypothesis that gold mining could be reducing methylmercury. Mercury is converted from Hg (II) to methylmercury by microbiological activity and such low methylmercury concentrations and percentages suggest a very low availability of mercury, possibly due to its binding to sulfur, abundant in the processed ore and hence in the mining effluents, and/or a strong inhibition of microbiological activity

due to the very high cyanide concentrations in these effluents. For the present study we returned to the region in 2009 to test the second hypothesis, measuring cyanide and bacterial activity in water along the Puyango river basin, as well as bacterial activity and mercury net methylation potentials in bottom sediments, in three reference stations upstream the mining area and four stations downstream.

2. Materials and methods

2.1. Study area and sampling sites

The Puyango River crosses southern Ecuador from NE to SW from the Andes' western foothills to the border with Peru, where it changes its name to Tumbes River, before finally reaching the Pacific Ocean (Fig. 1).

The Upper Basin of the Puyango includes the Zaruma and Portovelo mining and urban centers (stations 4) and the reference sampling stations upstream (stations 1 to 3): it is a temperate humid region. The Middle Basin includes Puyango Viejo and Marcabelf and it is a sub-tropical humid region, while the Lower Basin (Gramadal-Las Vegas and Chaguarhuaycu) is a tropical semi-arid region. The Upper Basin has around 42,000 inhabitants while the middle and lower parts of the Basin are scarcely populated. The river is an important source of fish and drinking water for communities living in the semi-arid Lower Basin while the towns of Zaruma and Portovelo in the upper basin draw their drinking water from small Puyango tributaries. Agriculture and cattle are the predominant economic activities in the Lower and Middle Basin, aside from gold mining. The reference stations 1 to 3 are almost pristine and range 1550 to 1820 m in altitude with little or no agriculture upstream. The waters carry very little suspended particulate matter and support a varied fauna of birds, macroinvertebrates and fish. In contrast, stations 4 and 5 are under the direct influence of untreated urban sewage and of mining effluents, showing very high levels of particulate matter and cyanide, Pb, Cd, Mn and Hg among other metals, leading to a severe reduction in the density and diversity of the aquatic fauna. The latter parameters

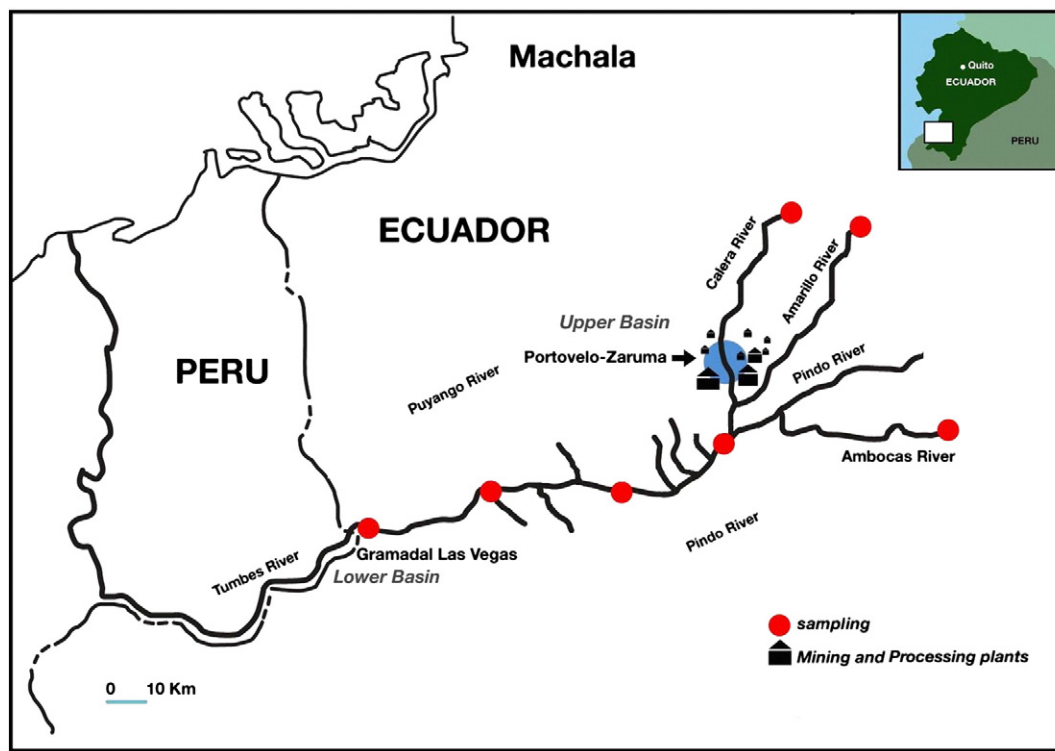


Fig. 1. Map of the study area and sampling stations, Puyango river basin, Ecuador.

increase only in farthest downstream station 7, at 155 m of altitude on the semi-arid coastal plain. Our sampling stations covered a 145 km stretch of the Puyango River basin. Inventories of benthic macro-invertebrate river fauna at background sites by [Tarras-Wahlberg et al. \(2001\)](#) showed the existence of 17 taxa in the upper Calera, and 13–23 taxa in uncontaminated stretches of the Pindo river but even as far as 160 km downstream the mining area only 5 taxa were found.

[Fig. 2](#) shows the altimetric profile of the Puyango basin, illustrating its high turbulence – and therefore turbulence – in the higher and middle basin and [Fig. 3](#) shows the typical dry season pattern for suspended particulate matter load in water across the study region, as measured during the dry season of year 2000 by [Betancourt et al., 2005](#). The different measurements described herein were made in the 2009 dry season.

2.2. In-situ measurement of cyanide in water

Free cyanide was measured in-situ, immediately after sampling, on a Hach field spectrophotometer model DR 2010 using the manufacturer reagent kit (Pyridene-Pyrazolone method, Hach reference 8027). This method is based on the chlorination of cyanide and the subsequent reaction of the product with a mixed solution of pyridinepyrazolone to form a stable complex dye. The method can be used to quantify total cyanide if samples are submitted to a previous distillation step. This was not possible in our field conditions, therefore the presented data refer to free and/or simple cyanides only.

Measurements were made both on unfiltered and on 0.22 µm-filtered water samples. The detection limit of the method was 1 µg·L⁻¹. Measurements of successive aliquots of the same sample of unfiltered or filtered water deviated by less than 2%. Free cyanide concentrations in the first two stations downstream the processing plants region were so high that a 20 to 50 fold sample dilution with distilled water was required to fall within the measuring range.

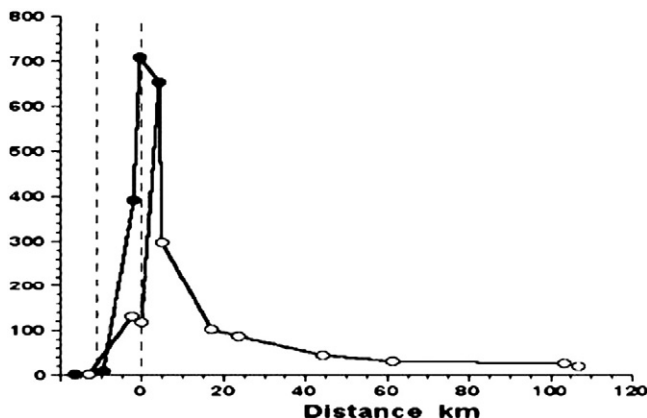


Fig. 3. Variation of the suspended particle load (mg·L⁻¹) along the Puyango river study area, November 2000. From [Betancourt et al. \(2005\)](#).

2.3. Bacterioplankton activity essays

The procedure for bacterial activity measurements was based on the method described by [Kirchman et al. \(1985\)](#), modified by [Miranda et al. \(2007\)](#). It consists of measuring the incorporation of radiolabeled leucine into bacterial protein over time ([Kirchman et al., 1985](#)). Unfiltered water samples of 1 mL were incubated in the dark for 1 h with 20 µL of a ³H-leucine solution with a specific activity of 2020 mCi·mmol⁻¹ and a leucine final concentration of 100 nM. For each sampling point, 2 controls killed with 50 µL of 37% formol and 4 samples were incubated. Incubations were stopped by addition of 50 µL of 37% formol and samples and controls were kept frozen until protein extraction.

2.4. Bottom sediment bacterial activity essays

Flocculent surface bottom sediments were sampled with a peristaltic pump and assayed immediately after sampling, by mixing 100 µL of

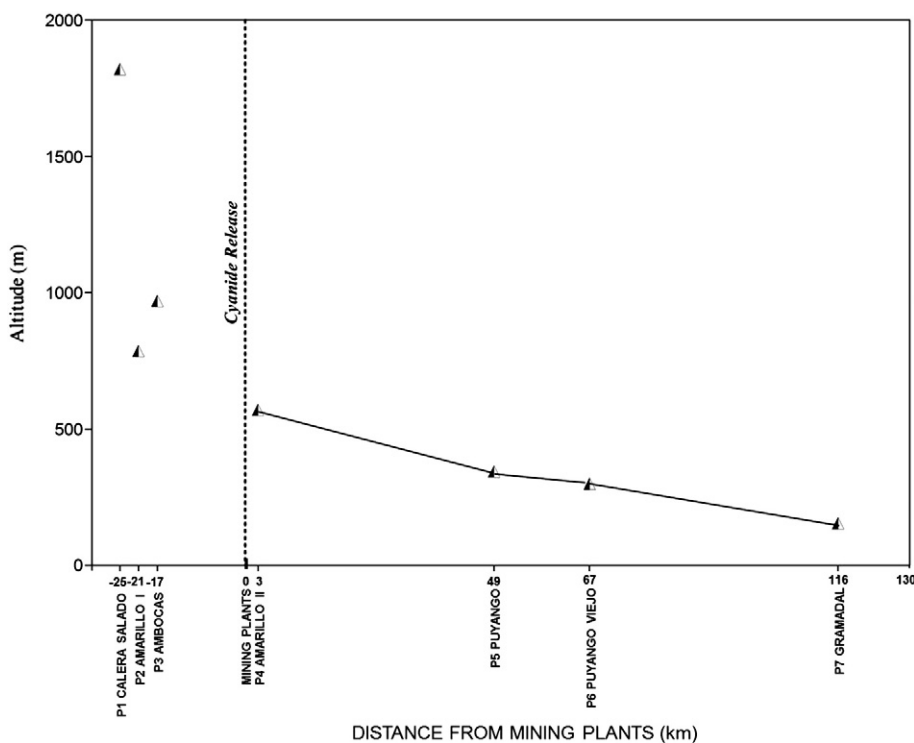


Fig. 2. Altimetric profile of the Puyango river basin.

sediment suspension with 900 μL of river water previously filtered in 0.22 μm membrane filters. Essays were started by the addition of 20 μL of a ^3H -leucine solution with a specific activity of 20 $\text{mCi}\cdot\text{mmol}^{-1}$ and a leucine concentration of 10 μmol . The other incubation conditions were as described above for water essays.

2.5. Protein extraction from water and sediment samples

The bacterial radiolabeled protein extraction was done as described by Miranda et al. (2007) with slight changes in water and sediment sample extraction procedure, as follows.

2.5.1. Water samples

After the incubation, 60 μL of 100% Trichloroacetic Acid ($\pm 5\%$, final concentration) was added to the microcentrifuge tubes for the precipitation of the proteins. The samples were kept in room temperature for 30 min and then centrifuged (14,000 rpm, 10 min). After centrifugation, the supernatant was aspirated carefully. Then 1 mL 5% TCA was added to the pellet, followed by homogenization for 10 s in a vortex, centrifugation and supernatant removal as above. A last clean-up step consisted of adding 1 mL ice-cold 80% ethanol, homogenization and centrifugation as above. The supernatant was aspirated and 1 mL of a scintillation cocktail was added to the samples. After 2 days in the dark, the samples were radioassayed by liquid scintillation counting (Perkin Elmer Tricarb 2800). Dpm data, corrected for quenching, were used to calculate bacterial carbon production ($\mu\text{gC}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$).

2.5.2. Sediment samples

After the end of incubation samples were sonicated in an ultrasonic bath for 10 min to detach bacterial cells. After sonication, the samples were centrifuged (2500 rpm, 15 min). The supernatant (900 μL) was recovered, transferred to new tubes and 60 μL of 100% Trichloroacetic Acid ($\pm 5\%$, final concentration) was added. The samples were kept in room temperature for 30 min and then centrifuged (14,000 rpm, 10 min). After centrifugation, the supernatant was aspirated carefully. Then 1 mL 5% TCA was added to the pellet, followed by homogenization for 10 s in a vortex, centrifugation and supernatant removal as above. A last clean up step consisted of adding 1 mL ice-cold 80% ethanol, homogenization and centrifugation. The supernatant was aspirated and 1 mL of a scintillation cocktail was added to the samples. After 2 days in the dark, the samples were radioassayed by liquid scintillation counting (Perkin Elmer Tricarb 2800). Dpm data, corrected for quenching, were used to calculate bacterial carbon production ($\mu\text{gC}\cdot\text{mg wet weight}\cdot\text{h}^{-1}$).

The relative standard error in the analysis of 4 water or sediment aliquots was respectively <20 and $<30\%$ of the average.

2.6. Methylmercury production essays in sediments

Hg methylation potentials were measured by means of a simplified radiochemical technique consisting of in-situ sample incubation with low amounts of $^{203}\text{Hg}^{2+}$ and extraction of formed Me^{203}Hg in toluene and quantification by liquid scintillation counting (Guimaraes et al., 1995). Fresh sediment samples, equivalent to 0.5 g dry weight were incubated in the dark with 30 mL of river water, in acid-washed Teflon® screw-capped 40 mL tubes. All incubations started in the field within a few hours after sampling. Duplicates and an acidified control, with 1 mL of 4 N HCl, received ^{203}Hg , added as $^{203}\text{HgCl}_2$, obtained from Eckert-Ziegler, USA, and corresponding to 24 ng Hg·g sample dry weight $^{-1}$. The ^{203}Hg stock solution was prepared in deionized water and kept at near 0 °C. Prior to each incubation series its activity was measured on a portable liquid scintillation detector (Triathler Hidex, model 425-004) and the added volumes of the ^{203}Hg solution were corrected, if necessary, to keep addition levels at 3500 dpm. After 24 h of incubation, Hg methylation was stopped by addition of 1 mL of 4 N HCl and samples

were frozen until MeHg extraction, performed within 15 days after incubations. Me^{203}Hg was leached with 4 mL of 3 M NaBr in 11% H_2SO_4 and 1 mL of 0.5 M CuSO_4 : after five minutes of shaking, the samples were centrifuged and the supernatant (~ 25 mL) was transferred to 60 mL glass separatory funnels and shaken for 15 min with 10 mL of scintillation cocktail (7 g·L $^{-1}$ POP (2,5-diphenyloxazole) and 1 g·L $^{-1}$ POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene] in 1 L of toluene). The aqueous phase was discarded and the overlying toluene layer transferred to glass tubes for centrifugation at 3000 rpm for 5 min. The clean toluene was shaken in a vial with 0.5 g of anhydrous Na_2SO_4 to remove traces of water containing inorganic ^{203}Hg and transferred to clean scintillation vials for measurement on a Perkin Elmer Tricarb 2800 liquid scintillation detector. Dpm data, corrected for decay, extraction efficiency and quenching were used to calculate Me^{203}Hg as a percentage of total added $^{203}\text{Hg}^{2+}$. The relative standard error for measurements on triplicate samples was $\sim 20\%$.

3. Results

As expected, free cyanide was not detectable ($<1 \mu\text{g}\cdot\text{L}^{-1}$) in unfiltered water from the three upstream stations and reached 280 $\mu\text{g}\cdot\text{L}^{-1}$ in the most impacted station situated 2.5 km downstream the last cyanidation plant (Fig. 4). Despite the strong declivity of the river basin, as illustrated in Fig. 2, and hence the high turbulence of the river, free cyanide is still at 48 $\mu\text{g}\cdot\text{L}^{-1}$ at station 6, ~ 80 km downstream the source, and becomes undetectable only after further 35 km, indicating that the high river turbulence was not enough to oxidize all free cyanide.

At stations 4, 5 and 6, in addition to unfiltered water, free cyanide was also measured in samples filtered through 0.2 μm membrane filters and in the three locations 50% of free cyanide in unfiltered water was in the dissolved phase, therefore readily bioavailable. Our data suggests that during the dry season, when dilution of mining effluents is reduced and mining activity increased, cyanide in water is far above suggested limits for approximately 100 km downstream the mining areas. Environment Canada (1995) suggests a 24 h maximum of 3.5 $\mu\text{g}\cdot\text{L}^{-1}$ free cyanide and the United States Environmental Protection Agency (1988) a limit of 5.2 $\mu\text{g}\cdot\text{L}^{-1}$ total cyanide. If the Puyango river water 2.5 km downstream the last cyanidation plant was considered as a mining effluent, its cyanide concentration would represent 14% of the upper limit set for this compound in the current Canadian metal mining effluent regulations (Minister of Justice, Canada, 2002). Our results are comparable to those reported by Tarras-Wahlberg et al. (2001) in the same river basin. The latter also measured cyanide during the raining season, finding lower concentrations but still often above the limits at 60 km downstream. In our data, a marked decrease in free cyanide is found between stations 6 and 7, from 48 to 2 $\mu\text{g}\cdot\text{L}^{-1}$. These stations are in the coastal plain and the longer river water transit time, combined with warmer temperatures, probably favors cyanide decomposition, due to degradation by bacteria and photochemical processes. Total cyanide was not measured in this work and the method we used is unable to recover cyanide that is complexed with metals, including Hg, or other compounds, so that total cyanide in the Puyango river is certainly well above the figures presented here.

Bacterial activity in water along the Puyango river basin shows contrasting patterns when expressed on sample volume basis (Fig. 5 A) or sample amount of suspended particles (Fig. 5 B).

When expressed on a volume basis, the data suggest an increase in activity in the stations downstream the mining area, but this is misleading as most bacteria in water are attached to organic or inorganic particles and the activity per cell is much higher in attached than in free bacteria (Haglund et al., 2002). Consequently, the graph A expresses the huge increase in suspended sediment load and urban waste introduced in the river just upstream station 4, as seen in Fig. 3, rather than a variation in activity per bacterial biomass unit, a parameter that was unfortunately not measured in this study. In

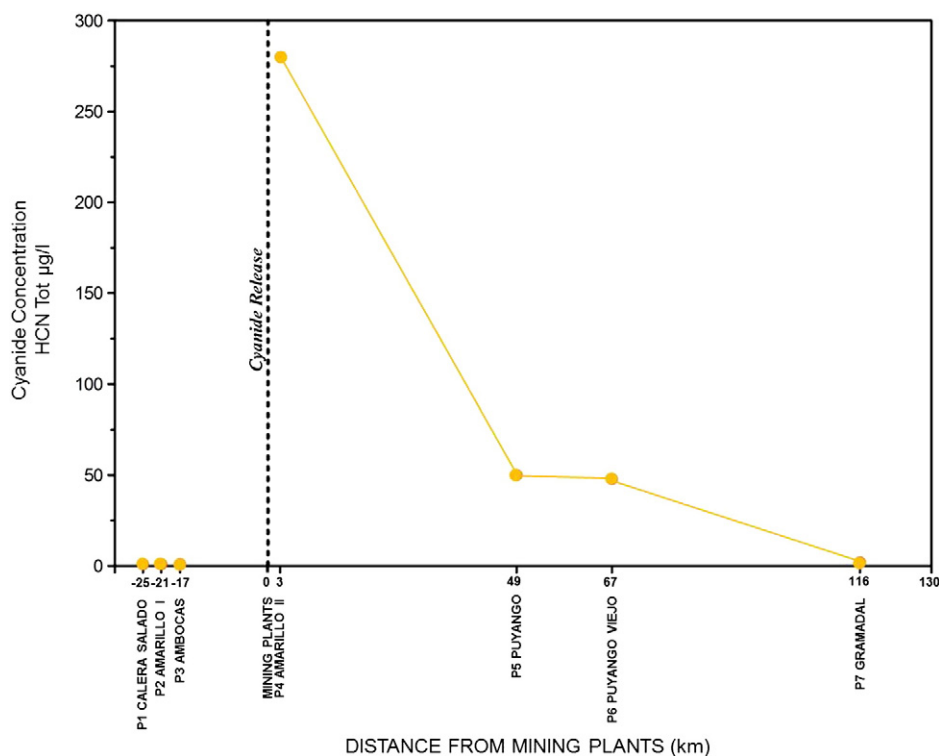


Fig. 4. Total cyanide ($\mu\text{g}\cdot\text{L}^{-1}$) in unfiltered water of the Puyango river, measured by in-situ spectrophotometry.

contrast, the graph B, describing activity expressed on basis of the amount of suspended particles, shows a steep decrease in activity in stations downstream the mining area, consistent with the intense point sources of toxic mining waste, especially cyanide. As we did not measure the suspended particles loads in this study, the data in graph B were calculated using the data from a 2004 sampling campaign done during the same period of the hydrological cycle, i.e. the dry season.

Hg methylation potentials and bacterial activity in sediments vary in the same fashion from station to station, with net methylation potentials of up to 22% in reference stations, falling to <0.2% in the two first stations downstream the processing plants, and reaching 19% in the most downstream station (Fig. 6).

Bacterial production followed the same pattern, though it remained detectable in stations 4 and 5, where net methylation was undetectable. Hg methylation potentials and bacterial production in sediments presented a significant positive correlation ($R^2 = 0.68$, $p < 0.022$, Fig. 7).

Downstream the processing plant area, the graphs for bacterial activity and net methylation in sediments are almost a mirror image of the one for cyanide in water, that in turn has similarities with the ones observed in the same season for metals in suspended solids or bottom sediments by Tarras-Wahlberg et al. (2001) and Betancourt et al. (2005). The similarity is due to their common source and to the probable formation of cyanide/metal complexes. Cyanide is a strong metal ligand, which explains its use in the mining industry. However, released together, cyanide and metals drift downstream where cyanide will eventually decompose and release the metals.

The very similar longitudinal profiles of net Hg methylation and bacterial activity in sediments advocates for a microbiological Hg methylation process. The methylation potentials measured here in the upper basin stations are higher than found in all previous measurements in sediments from different aquatic systems in Brazil and Bolivia (Guimaraes et al., 2000) however the latter did not include temperate humid systems such as the ones in the upper Puyango basin.

4. Discussion

We used two radiotracer techniques and a conventional field spectrophotometric one to simultaneously measure free cyanide, bacterial activity in water and sediment and net mercury methylation in sediments along a 140 km stretch of the Puyango river basin. Previous work by our team (Betancourt et al., 2005) and other researchers has shown the significant impact of mining on this river basin, due to inputs of cyanide and of high loads of suspended material with high concentrations of metals such as Hg, Cd, Pb, Cu, Mn and Zn. The apparent paradox observed by Roulet et al. (2006) of high total Hg concentrations but very low MeHg concentrations in stations downstream the mining area lead us to investigate the impact of mining effluents on bacterial activity and microbial mercury methylation on the same river stretch. Our data show the suppression or drastic decrease of bacterial activity and mercury methylation in water and sediment downstream the mining area, where free dissolved cyanide concentrations are highest, and recovery of these parameters in the stations at greater distances from the pollution sources, where cyanide is lower or undetectable. This suggests that cyanide toxicity for bacteria reduced their activity and consequently microbial mercury methylation. However, though the reduced bacterial activity alone could explain the reduction in mercury methylation, the latter can also be reduced by factors other than cyanide toxicity for microbes. The formation of both cyanide–Hg complexes and of sulfur–Hg complexes reduces Hg (II) availability for methylation. Accordingly, Tarras-Wahlberg et al. (2001) found that though sediments were enriched in mercury throughout the Puyango middle and lower basin, the proportions of reactive Hg were very low. Roulet et al. (2006) showed that Hg and S correlated positively in sediments and suspended particles downstream the mining areas of the same river stretch as herein, both in the dry and wet seasons (Fig. 8). Another factor that may contribute to a low availability of Hg downstream gold mining fields is the formation of micro-droplets of metallic Hg onto hydrous iron oxides and clay minerals (Meech et al., 1998). In a previous unpublished work in the region we found less total Hg in the piscivorous fish

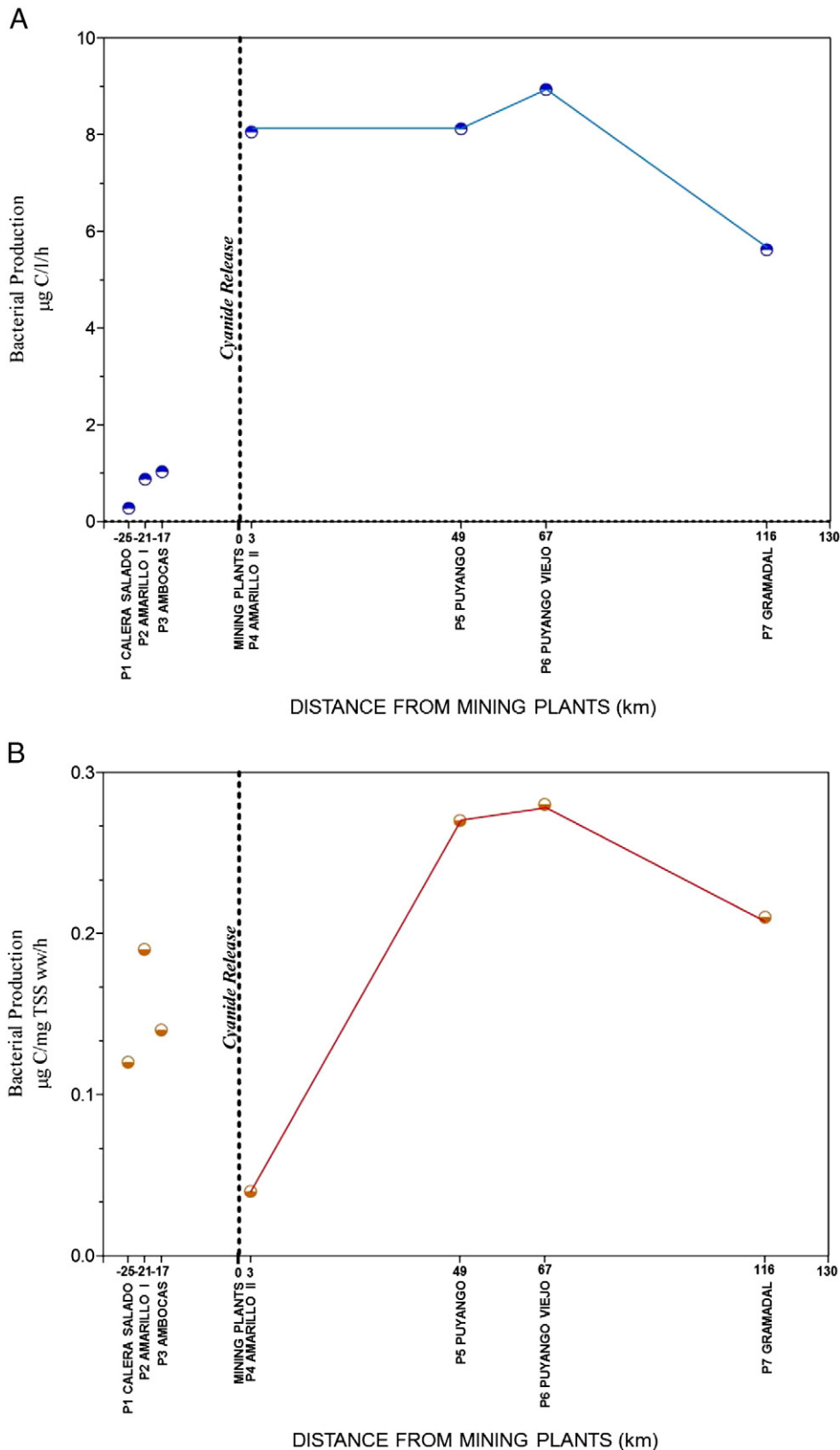


Fig. 5. Bacterial activity in water of the Puyango river expressed on a volume (graph A, $\mu\text{gC}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$) and SPM mass (graph B, $\mu\text{gC}\cdot\text{mg}\text{ww}^{-1}\cdot\text{h}^{-1}$) basis.

Brycon atrocaudatus in the middle Puyango basin ($0.8\mu\text{g}\cdot\text{g}^{-1}$ wet weight, $N=8$) than in the lower basin ($1.5\mu\text{g}\cdot\text{g}^{-1}$ wet weight, $N=14$), (Barriga R et al. in prep.). Unfortunately, no data are available so far on the speciation of Hg in fish from the Puyango river, including MeHg or other organic and inorganic Hg compounds, in particular

cyanide–Hg complexes, that may be directly bioaccumulated (Velásquez-López et al., 2011).

In the local ore, gold is indeed associated to S-rich minerals, but the presence of carbonate rocks and the low residence time of mining tailings on the river banks do not favor the production of acid mine

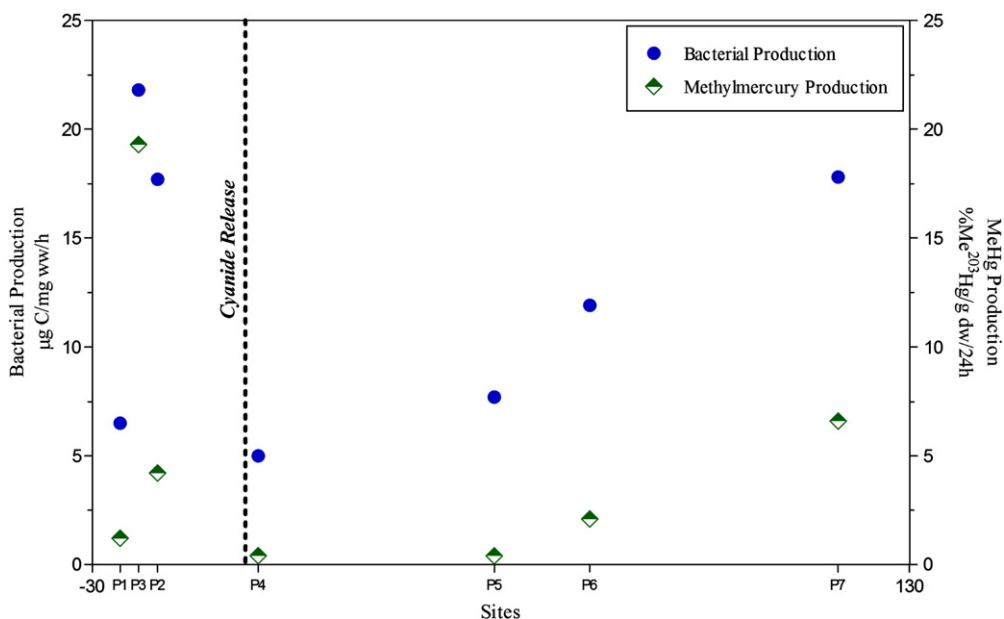


Fig. 6. Hg methylation potentials (% of total ²⁰³Hg·g dry weight⁻¹·day⁻¹) and bacterial production (µg·C·mg wet weight⁻¹·h⁻¹) in bottom sediments along the Puyango river basin.

drainage, and previous work in the region by Roulet et al. (2006) showed that water pH was lower (6.3–7.0) in reference upstream points than 2.5 to 110 km downstream the mines (6.6–8.1). In the absence of prevalent acid mine drainage, cyanide alone could explain the eradication of the aquatic biota in stations downstream the mining area but the impact of increased metal concentrations and of the direct dumping of nearly a million t of tailings per annum in the river certainly plays a relevant role. In fact, in gold mining areas where amalgamation or processes other than cyanidation are used for gold recovery, the finding of significant MeHg concentrations and MeHg/total Hg ratios downstream the mines is rather the rule than the exception, as reported by Gray et al. (2002) in Suriname, Gray et al. (2004) in Almaden, Spain, Stamenkovic et al. (2004) in Nevada, USA, Johnson et al. (2009) in California, USA, and Boudou et al. (2005) in French Guyana, to cite a few. Even in active or historical mining sites where sulfide concentrations are higher, reducing Hg reactivity, enhanced methylation may be found further downstream (Domagalski, 2001).

In our study site, as put by Tarras-Wahlberg et al. (2001) “Elucidating the relative severity of impact of the mining related contaminants is made difficult by the fact that cyanide, mercury and metal rich tailings are present together in the affected rivers”. However,

the same authors measured cyanide and metals in two different branches of the Puyango basin with similar metal concentrations but with cyanide concentrations differing by one to two orders of magnitude and suggest that cyanide rather than metals would explain the eradication of biota on long river stretches. Our data showing the strong inhibition of bacterial activity downstream the mining area gives additional support to this hypothesis.

5. Conclusion

The inhibition of MeHg formation on a long river stretch downstream a gold mining area is a peculiar finding that reflects the interplay of ecosystem characteristics and human activities, with implications for health. It may explain, together with the low fish intake, the low total mercury in human hair in the basin (mean 1.17 µg·g⁻¹). Though

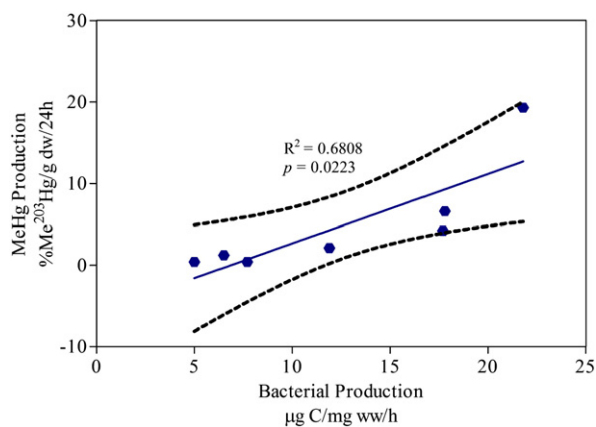


Fig. 7. Hg methylation potentials plotted against bacterial production, sediments of the Puyango river basin.

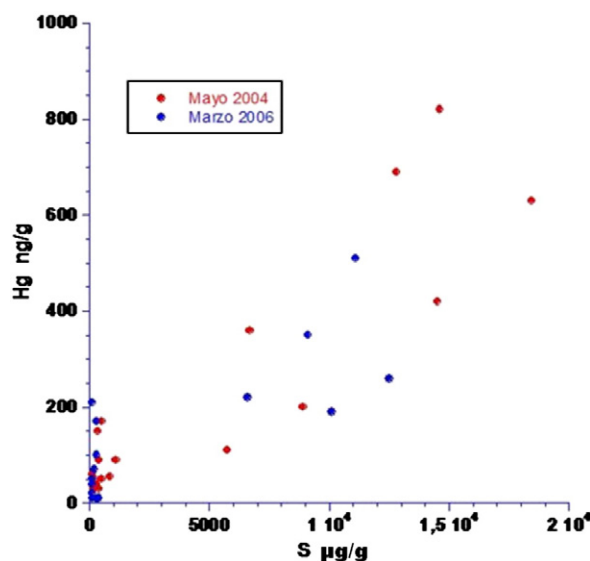


Fig. 8. Total Hg and Sulfur in suspended particles along the Puyango river Basin in the dry and wet seasons. From Roulet et al. (2006).

toxic by itself, cyanide ironically seems to reduce mercury toxicity, by reducing MeHg formation and consequently mercury bioavailability to the local population.

However, we showed here that when reaching the coastal plain, the Puyango river shows no detectable cyanide in water and that mercury methylation in its sediments returns to levels comparable to the ones in pristine stations. In contrast with pristine stations, the coastal plain sediments and suspended particles are rich in mercury, and further downstream, intensive shrimp farming is the main economic activity, in a potential conflict between two activities of comparable economic importance to Ecuador.

Acknowledgments

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This work is dedicated in the memory of our colleague and very close friend Marc Roulet, (1967–2006), a distinguished biogeochemist at Institut de Recherche pour le Développement, La Paz, Bolivia, who enthusiastically participated in this project.

References

- Astudillo C. The sweat of the sun: history of gold mining, Ediciones La Tierra; 2007. 190 pp. (In Spanish).
- Betancourt O, Narvaez A, Roulet M. Small-scale gold mining in the Puyango River Basin, Southern Ecuador: a study of environmental impacts and human exposures. *Ecohealth* 2005;2:323–32.
- Boudou A, Maury-Brachet R, Coquery M, Durrieu G, Cossa D. Synergic effect of gold mining and damming on mercury contamination in fish. *Environ Sci Technol* 2005;39:2448–54.
- Domagalski J. Mercury and methylmercury in water and sediment of the Sacramento River Basin, California. *Appl Geoch* 2001;16:77–91.
- Environment Canada. Interim sediment quality guidelines. Ecosystem Conservation Directorate. Ottawa: Evaluation and Interpretation Branch, Guidelines. Division; 1995. p. 9.
- Gray JE, Labson VF, Weaver JN, Krabbenhoft DP. Mercury and methylmercury contamination related to artisanal gold mining, Suriname. *Geophys Res Lett* 2002;29:2105.
- Gray JE, Hines ME, Higuera PL, Adatto I, Lasorsa BK. Mercury speciation and microbial transformations in mine wastes, stream sediments, and surface waters at the Almaden Mining District, Spain. *Environ Sci Technol* 2004;38:4285–92.
- Guimarães JRD, Malm O, Pfeiffer WC. A simplified radiochemical technique for measurement of net mercury methylation rates in aquatic systems near goldmining areas, Amazon, Brazil. *Sci Total Environ* 1995;175:151–62.
- Guimarães JRD, Meili M, Hylander LD, Castro e Silva E, Roulet M, Mauro JBN, et al. Net mercury methylation in five tropical flood plain regions of Brazil: high in the root zone of floating macrophyte mats but low in surface sediments and flooded soils. *Sci Total Environ* 2000;261:99–107.
- Haglund AL, Törnblom E, Boström B, Tranvik L. Large differences in the fraction of active bacteria in plankton, sediments, and biofilm. *Microb Ecol* 2002;43:232–41.
- Johnson BE, Esser BK, Whyte DC, Ganguli PM, Austin CM, Hunt JR. Mercury accumulation and attenuation at a rapidly forming delta with a point source of mining waste. *Sci Total Environ* 2009;407:5056–70.
- Kirchman DL, Knees E, Hodson RE. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Appl Environ Microbiol* 1985;49:599–607.
- Meech JA, Veiga MM, Tromans D. Reactivity of mercury from gold mining activities in darkwater ecosystems. *Ambio* 1998;27:92–8.
- Minister of Justice, Canada, metal mining effluent regulations, SOR/2002-222. Available at <http://laws-lois.justice.gc.ca>.
- Miranda MR, Guimarães JRD, Coelho-Souza AS. [³H] leucine incorporation method as a tool to measure secondary production by periphytic bacteria associated to the roots of floating aquatic macrophytes. *J Microb Meth* 2007;71(1):23–31.
- PRODEMINCA. Evaluation of impacts in the mining district of Zaruma–Portovelo and the Puyango river basin. Proyecto Desarrollo Minero y Control Ambiental, Prode-minca. Quito: Swedish Environmental Systems. Ministerio de Energía y Minas del Ecuador; 1998. 212 pp. (In Spanish).
- Roulet M, Cueva E, Sandoval J, Iñiguez V, Betancourt O. Gold mining in the Andes (Ecuador) can lower MeHg in sediments. Coloquio Internacional sobre la Biogeoquímica de los Metales en el Medio Ambiente y su Impacto en la Salud de las Poblaciones Humanas, IRD/UMSA, La Paz, Bolivia; 2006. Nov. (In Spanish).
- Stamenkovic J, Gustin MS, Marvin-DiPasquale MC, Thomas BA, Agee JL. Distribution of total and methyl mercury in sediments along Steamboat Creek (Nevada, USA). *Sci Total Environ* 2004;322:167–77.
- Tarras-Wahlberg NH, Flachier A, Lane SN, Sangfors O. Environmental impacts and metal exposure of aquatic ecosystems in rivers contaminated by small scale gold mining: the Puyango River basin, southern Ecuador. *Sci Total Environ* 2001;278:239–61.
- United States Environmental Protection Agency. Ore mining and dressing point source category effluent limitations guidelines, 40 CFR 440; 1988.
- Veiga M.M. Personal communication, May 2011.
- Velasquez-Lopez PC, Veiga MM, Hall K. Mercury balance in amalgamation in artisanal and small-scale gold mining: identifying strategies for reducing environmental pollution in Portovelo–Zaruma, Ecuador. *J Clean Prod* 2010;18:226–32.
- Velasquez-López PC, Veiga MM, Klein B, Shandro JA, Hall K. Cyanidation of mercury-rich tailings in artisanal and small-scale gold mining: identifying strategies to manage environmental risks in Southern Ecuador. *J Clean Prod* 2011;19:1125–33.