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Impacts of anthropogenic activities on the contamination of a sub watershed of Lake Titicaca. Are antibiotics a concern in the Bolivian Altiplano?

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Abstract

The Titicaca Lake is the most important water resource over the Andean plateau and the ecological equilibrium of this region is nowadays perturbed by recent changes in land use and management practices. The Katari watershed encompasses mining area, cities representing over 1.2 million habitants, and agricultural zones before ending in Cohana bay in the Titicaca Lake. Cohana Bay is known to be one of the most eutrophic bay of the Titicaca Lake. The objective of the study was to evaluate the impact of anthropic activities along the watershed on the river quality and on the bacterial diversity. Both mining activities and release of wastewater in river systems impacts greatly the surface water quality, with level of As exceeding limits for drinking water, and phosphate over the European guidelines for bad quality rivers. Antibiotic from the sulfonamide family was detected in the watershed in high concentrations downstream of the two main cities and bacterial resistance occurred in nearly all the sampled water points.

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Keywords: antibiotics; nutrients; trace metallic elements; bacterial resistance.

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1. Introduction

The Titicaca Lake constitutes the most important water resource on the Altiplano and the most elevated in the world. The ecosystems present inside the lake and in the surrounding watersheds, as well as human activities developed since millenaries, adapted themselves to very constraining climate and soil conditions. The ecological equilibrium of this region is nowadays perturbed by recent changes due to an important urban demographic growth, to the entry of the society in modernity and to the transformations of usages and practices at local and global scales. For millennia, the edges of Lake Titicaca have been subject to intense mining leading to trace metal elements contamination [1]. The Katari watershed, located in the northern part of the Altiplano includes mining zones, as well as urban, industrial and agricultural area. Salvarredy-Aranguren et al. [2] studied the mining related contamination in the Milluni Valley, located in the north-east part of the Katari watershed and they found that surface water were strongly contaminated by the acid mine drainage from mining operations in the area. The ecological balance of the watershed and Titicaca Lake is also disturbed by domestic and industrial wastewater discharges from rapidly growing urban areas. Katari watershed encompasses El Alto city, the biggest city in the Altiplano, which is a very good example of a quick developing city sensible to environmental changes. El Alto population increased from 95,000 habitants in 1976 to around 1.2 million according to the last census without any land planning and regulation. The wastewater collect and treatment system of this city are insufficient [3] and cover only about 50% of this fast growing city, which causes wastewater discharges directly into the rivers with subsequent impacts on the quality of the surface waters [1]. The outlet of Katari watershed, Cohana Bay, has the waters among the most eutrophic of the lake [4]. Antibiotics were already reported to have adverse effect on human health in Bolivia [5] but no study has been performed on their presence in the river systems and their impact on bacterial community. This present study aims (i) to document the occurrence of trace metals, nutriments, and antibiotics in the Katari watershed and to (ii) evaluate the impact of contamination on bacterial resistance along the watershed. It is the first study of its kind to provide baseline information on the multi-contamination risks of water resources around the Titicaca Lake.

2. Materials and methods

2.1. Studied site

The Katari watershed is about 2022 km^2. Only a part of the Katari watershed was sampled, the most populated, (Fig. 1), which represents 40% of the whole Katari watershed. The watershed is dominated by summits reaching 6000 m asl in the Eastern Andes (Cordillera Real, Huayna Potosi, 6088 m asl). Four main rivers flow through the considered part of the watershed: the Katari, Seco, Seke and Pallina rivers. The Seke and Seco rivers take their source in the Milluni Valley where a network of diffuse runoff and wetlands can be observed during the wet season. Seke river discharges into Milluni lakes, which receive mining drainage [2] and serve also as the main reservoir of drinking water for la Paz city. Downstream, Seke and Seco rivers flow through El Alto (1.2 million habitants) and Viacha (80000 habitants), where treated and untreated urban waters are discharged into it. These rivers discharge into the Rio Pallina, itself discharging into the Katari river. Finally the Katari river runs through rural areas and discharges into Titicaca Lake in the Cohana bay.

The climate of the region is semi-dry and cold as determined by the Intertropical Convergence Zone and by its high altitude (over 3800 m). The region has extreme diurnal variations of climatic factors such as solar radiation, temperature and moisture deficit. Rainfall is very low with an average annual precipitation of 492-620 mm/year, concentrated from December to March.

2.2. Sampling and analytical procedures

Surface waters were sampled in June 2012 (dry season) and February 2013(wet season) at 12 points along the watershed (Fig. 1a). 5 AM and 5 AV correspond to the entry and the exit of the Waste Water Treatment Plan (WWTP) at el Alto respectively: 5 AV is located just before the release of treated waste water in the Seco River. At each point, flow rate, pH, temperature and Electrical conductivity were measured *in situ*. Samples were taken for nutrients, antibiotics, and trace elements in dissolved phase, as well as trace elements in particulate phase and sediments. Filtration at 0.45µm if necessitated was performed *in situ* or the same day at the laboratory. Nutrients were analyzed by ionic chromatography for anions and by ICP AES for cations and trace metals. A screening of different antibiotics families (Sulfonamides, Penicillin G, Cyclines, Trimethoprim, Chloramphenicol) was analysed by HPLC MS/MS. The existence of bacterial resistance to sulfamethoxazol in water was evaluated by PCR (genes *Sul1*, *Sul2* and *Sul3*).

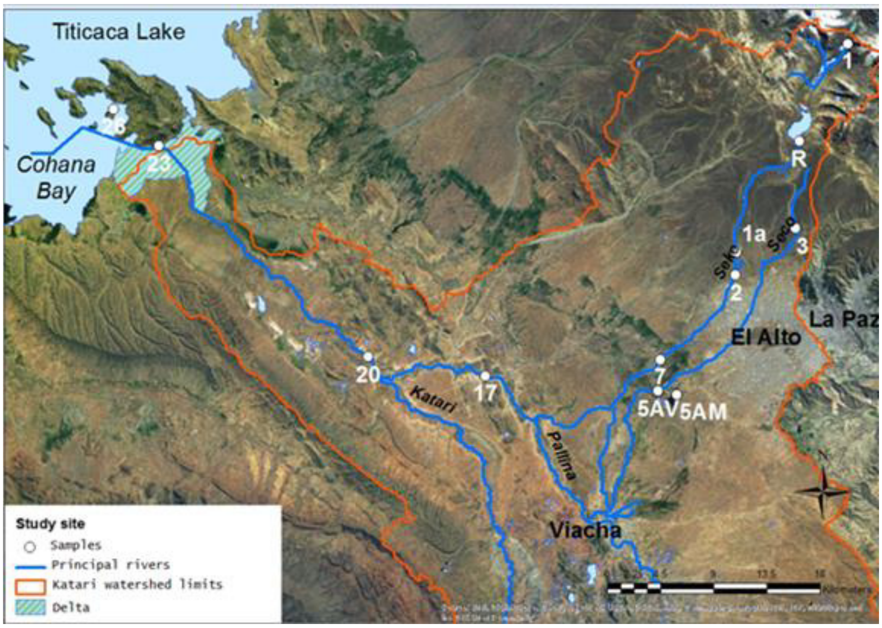

Fig. 1. Map of part of the Katari watershed and sampling sites

3. Results and Discussion

3.1. Nutrients concentration and trophic state indicators

During the dry season, the water samples upstream (4500-4700m a.s.l, points 1 and 3, Fig. 1) show the pattern of an unpolluted oxygenated watercourse with low aqueous Fe, Mn and phosphate (< limit detection) and low nitrate (< 1 mg L^{-1}). The rivers water quality changes entirely when entering the urban area. The appearance of two redox indicators, Fe and phosphate are observed (Fig. 2). Phosphate concentrations may be considered extreme, as they surpass the European guidelines for highly polluted river waters (2 mg L^{-1} PO_4^{-3}, European guidelines) by a factor of 10. Aqueous Fe, presumably present as Fe(II), is measured at 0.1 mg L^{-1}. These concentrations characterize eutrophic conditions which relates to oxygen depletion, algal and slime development, and high bacterial loads. This quality change is due to the high inputs of untreated waste waters entering rivers of low flow, i.e. low dilution and self-cleaning potential especially in the dry season. The discharge of the WWTP of El Alto represents 0.5 to 7 times the natural flow rate of the Seco river at the point of confluence, in wet and dry season respectively. Finally, the river water quality changes again at the entrance of Lake Titicaca: Phosphate and Fe are depleted. This decrease presumably relates to both, dilution and re-oxygenation effects.

During the wet season, river flow rates increases strongly. At the sampling dates they were multiplied by 50 to 300 between dry and wet season. This change in flow rate affects the water chemistry. Nutrients (NO_3^-, PO_3^{-3}) still distribute similarly in the watershed compared to dry season but at lower levels because of dilution (Fig. 2). Ammonium (data not shown), only measured in the wet season campaign, was only detected in the polluted urban area, confirming the eutrophic state of the water. In both wet and dry season, Chloride increases from almost < 1 mg L^{-1} upstream to ~ 100 mg L^{-1} at the entry of the urban area and ~200 mg L^{-1} after the confluence with the Pallina. Similarly, Sulfate concentrations are high between 30 and 120 mg L^{-1} and do not vary a lot between dry and wet season. Chloride and Sulfate concentrations remain high even in the estuary of the Titicaca Lake, where the salinity is high: the electrical conductivity ranges between 1200 and 1500 µS cm^{-1} [6]. The WWTP does not seem to have an impact on nutrients concentrations, as the concentration before and after are similar at least in wet season. In opposite, Fe and Mn concentrations distributes differently between wet and dry season. In the wet season, they are measured in the whole transect at concentrations ≤ 2 mg L^{-1} for Fe and ≤ 0.7 mg L^{-1} for Mn. In parallel, the presence of moderate dissolved oxygen is measured in all points. This co-presence of metals in reduced state and oxygen in the urban area is due to rapid oxygenation because of good mixing with high water flow. The presence of aqueous Fe, presumably Fe(II), in the top uncontaminated samples upstream the mining site and urban area is more difficult to explain. Eventually, sulfide to sulfate oxidation in local ore deposits reduces the abundantly present Fe oxides. This Fe(II) is thus leached to the stream during the wet season.

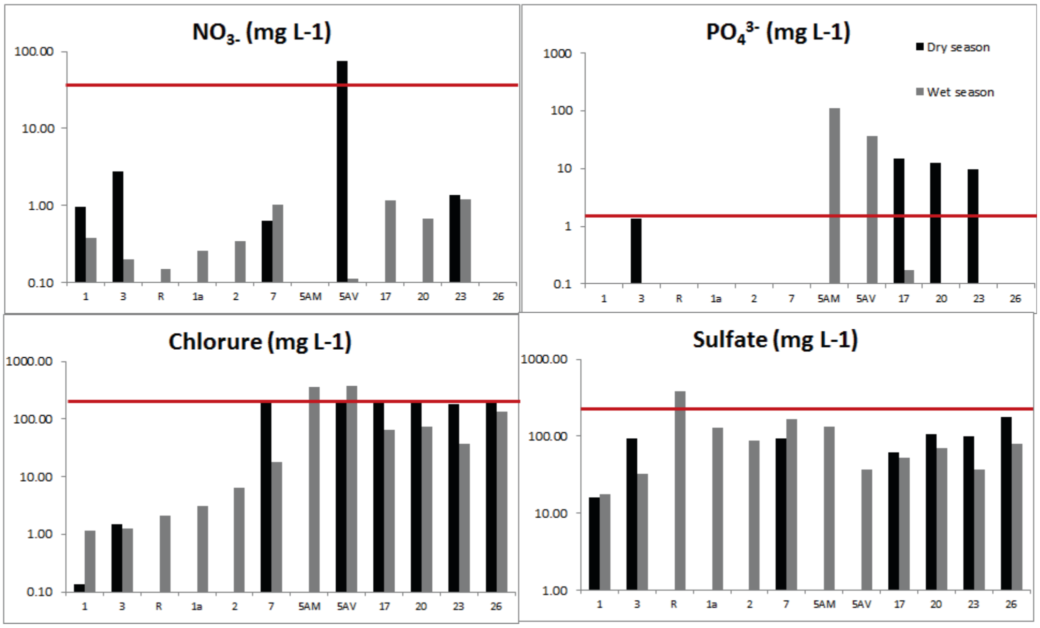

Fig. 2 : Evolution of nitrate, phosphate, sulfate and chloride along the watershed. Red line: European guidelines for bad quality river waters

3.2. Dissolved and particulate trace metal concentrations

The sampling point R was downstream of the Sn mining area and metallic elements are mainly in dissolved phase (Fig. 3) because of low pH (3.5). This change in pH relates to acid mine drainage, i.e. the acid generating oxidation of reduced anions such as sulfides during ore processing. Downstream the mining site, the pH rapidly re-equilibrates to neutral to basic values, this stands for a sufficient buffering pH effect of the water and minerals in contact with flowing water, or respectively a moderate impact of the mining activity on the water quality. Across the urban area (from points 1a to 7), the dissolved concentrations of Cd, Ni, Zn, As increase nearly twofold (Fig. 3). However, particulate concentrations are constant expect for Zn at point 7. Dissolved metallic elements may be generated by mining activities (point 3), waste landfills (above point 1a), and wastewaters (points 2 and 7). Levels of dissolved and particulate metallic elements increase after the WWTP (point 5AV). Particulate concentrations increase twofold and is due to flocculation processes in the ponds. Point 17 receives black water from El Alto and Viacha cities, and dissolved concentrations for As and Pb are measured similar to levels at the WWTP exit. There is an increase of particulate and dissolved concentrations between 20 and 23 showing impacts of anthropic activities in the Cohana bay. Ba, Cd, Cr, Cu, Mn, Ni, Zn, Al and Fe concentrations were greater in wet season (data not shown) in accordance with previous results[2], which is probably linked with leaching from rocks by percolating rain water and with the presence of acid mine drainage upstream of the Milluni lac reservoir area. The acid mine drainage results from contact of rainwater with crushed or broken rock that contains iron sulfide. Besides, Se and As concentrations were generally greater in dry season.

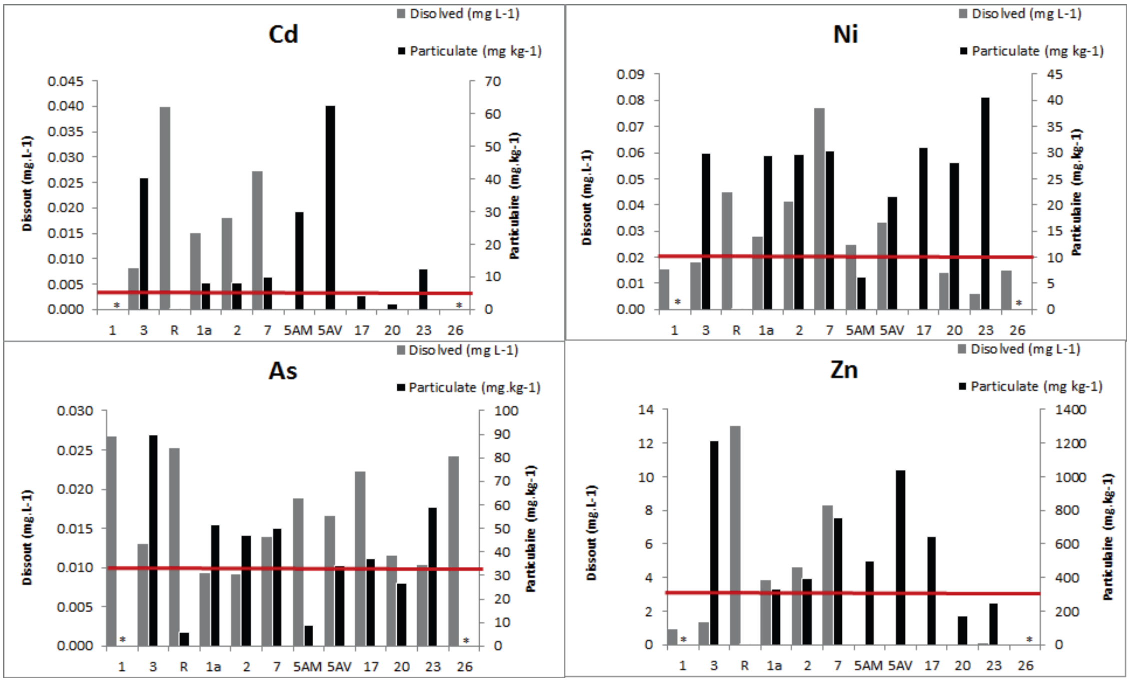

Fig 3. Evolution of Cd, As, Ni and Zn in dissolved and particulate phase in the wet season. * means that the concentration could not be analyzed because the particulate matter was not enough.

In regards with World Health Organization (WHO) water guidelines for human consumption, As is the metalloid the most of concern in the Katari which always exceeds threshold of 10µg L^{-1} (Fig. 3). Dissolved As is a residue from mining activities resulting from the oxidative weathering of arsenopyrite and is evenly widespread among the watershed. Cd, Ni, Zn and Pb overpass also their respective tolerable limit for water guidelines (0.003 mg L^{-1} for Cd, 0.02 mg L^{-1} for Ni (EU guideline), 3 mg L^{-1} for Zn and 0.01 mg L^{-1} for Pb) upstream the WWTP. Downstream the WWTP, the waters exhibit very low concentrations of dissolved Cd, Ni, Zn (Fig. 3) and Pb and metals reach the particulate fraction (same trends were observed for Cr, Cu, Sn, Mo, Co, data not shown). A hypothesis to explain this behavior is the enrichment in organic matter downstream the WWTP which complexes dissolved metal

3.3. Antibiotics and antibiotics resistance

The screening analyses were performed at points 3, 5AV, 17, 20 and 23. Sulfamethoxazol (SMX) and Trimethoprim (TMP) were the predominant antibiotics detected (Table 1). These two antibiotics are commonly used in the world, sometimes together, for illness treatment for humans and animals. SMX concentrations downstream of the two main cities of the watershed (8832 and 14624 ng L^{-1}) are much higher than concentrations detected in the Seine river in Paris [7] (3.6 to 18 ng L^{-1}) and in the Mekong river in Vietnam [8] (1720 ng L^{-1}). To date, no study shows that this level of concentration has adverse effect for human health. However, the main impact is the modification of biodiversity of indigenous soil and water bacterial population and possible development of bacterial resistance. The last report by WHO [9] globally reveals that antibiotic resistance impacts every region of the world and is now a major threat to public health. All sampling points present SMX resistance genes (gen *sul1* was the most frequently observed), even in sites located away from SMX sources (point 1). This could be due to the natural presence of metallic elements. Bacterial resistances to diverse metals and antibiotics are often genetically linked, suggesting that exposure to toxic metals may select for strains resistant to antibiotics and vice versa. The only site where not SMX resistance genes were found was the R site, located at the output of the Milluni water reservoir. At this site, the highly acidic water (pH = 3) can affect the development of bacterial communities.

Table 1. Main antibiotics analysed in 5 points of the watershed (ng L^{-1}). LD: detection limit, LQ: quantification limit

Antibiotic	3	5AV	17	20	23
Sulfamethoxazol	63	8832	14624	8893	86
Trimethoprim	31	2632	4474	28	26
Sulfathiazol	1.5	114	137	59	<LQ
Chloramhenicol	23	20	28	<LD	<LD
Sulfadiletoxine	3.4	<LQ	<LQ	<LD	20

4. Conclusions

In the Katari watershed, the release of wastewater in river systems has a great impact on water quality: nutrients, especially phosphate, exceed greatly levels for bad quality river water downstream of the two main cities. Trophic indicators such as dissolved Mn and Fe and oxygen depletion show that waters are eutrophic. The trophic state of Cohana Bay inside the Titicaca Lake is much better: the native plant *Schoenoplectus tatora*, commonly known as totora, growing in the Cohana Bay, is known to remove nutrients such as phosphorus and nitrogen from effluents [10]. Another major impact of wastewater release is the presence of antibiotics, especially SMX, in higher concentrations than in much bigger cities than El Alto. This leads to bacterial resistance to antibiotics in all the sampled points of the watershed except one. Sn mining site upstream of El Alto city releases As in concentrations higher than the WHO drinking water levels and its concentration stays high all along the watershed down to the Lake. Dissolved concentrations of other metallic elements (Cd, Zn and Ni) are high (over the drinking water standards) upstream the watershed, but decrease downstream because they sorbed on suspended sediments.

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