ANNEX 1. Final Technical Report

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1. INTRODUCTION

The Rio Hardy is located at the northeast corner of the state of Baja California, Mexico and is currently the main drainage of the Mexicali agricultural valley. Historically, the Rio Hardy was an arm of the Colorado River (Sykes, 1937) which flowed into the upper Gulf of California. Damming of the Colorado River from 1930 to 1960 substituted the natural river flows with agriculture return flows. Consequently, water quality and quantity at the Hardy is a major concern for local residents, stakeholders and the environment.

Las Arenitas Water Treatment Plant (WTP) was inaugurated on March 2007 by Pres. Felipe Calderón, it cost 240 million pesos (about 20 million dollars). The plant treats a large portion of the Mexicali sewage that formerly crossed the city and was released to the Salton Sea in California through the Rio Nuevo. The plant operates at half capacity treating 0.88 m^3/s (31 cfs) of Mexicali sewage, of which 0.55 m^3/s (19 cfs) began to be released to the Rio Hardy in October 2007. However, Las Arenitas WTP has not been well received by the Rio Hardy users. Since the beginning of operations, users and residents denounced foul odours, green coloration of water and several fish die-offs at the north of the river. This crisis peaked on January 2008, although complaints lasted all through 2008 (Reports from La Crónica newspaper, www.lacronica.com).

Our work consisted on monitor water quality at different points on the Rio Hardy and at Las Arenitas WTP. In this report we will analyze 2006 and 2008 water quality data, in order to provide a more comprehensive analysis of the situation at the Rio Hardy prior and after the operation of the plant, we will also look into possible solutions and recommendations.

2. OBJECTIVES

2.1. To sample water at 5 sites: Hardy north, Mosqueda, Comunidad Cucapa, Sonora and Ramona, which are used for recreation and Hardy north is where effluents are received.

These sites will be monitored monthly for general water quality parameters (total dissolved solids, phosphates, nitrates, oxygen, temperature, salinity, conductivity, pH and ORP) and for pathogens (*E. coli*)

2.2. To sample water, sediments and organisms after the Las Arenitas discharge for the analysis of metals, organochlorine pesticides and PCBs

2.3. To collect and analyze biomarkers of effect in sessile organisms collected after the operation of the plant.

3. RESULTS

3.1. Sampling sites

In 2006 we monitored 7 sites between the discharges on the north and the first tourist camp (Mosqueda) (Fig. 1 Table 1), this was made with the objective to detect a gradient in the number of *E. coli* and other contaminants, which in fact, was observed: higher concentrations at the top and lower at the last Hardy stations. However, for the 2008 sampling effort, we focused on recreational camps and the inflows at the top (Fig. 1 Table 1), so we added site 1, which is located inside Las Arenitas WTP, at the discharge of the oxidation lagoons (Photo 1, Annex 1). We also added site 2 (Puente Nayarit), which is the discharge of WTP effluents into Rio Hardy (Photos 2 and 3, Annex 1). At the time of the photo, flows were minimal due to suspension of deliveries from the plant due to complaints by Rio Hardy residents and users. And site 3 (Dren Durango) which is an important agricultural and urban drain (Photo 4, Annex 1). The tourist camps that we continued to monitor were Campo Mosqueda (Site 12), the largest tourist camp on the Rio Hardy; Comunidad Cucapa (Site 15), which is the pond on the river where the native Cucapa accustom to swim; Campo Muñoz (Site 17) which is where the Rio Colorado joins with the Hardy, when flows permit; and Campo Ramona (Site 18) which is the last tourist camp on the river, and is the site where many conservation and restoration projects are taking place. A new camp that was included in 2008, was Campo Sonora (Site 11), this is located on one side of the highway to San Felipe and is a separate backwater of the Rio Hardy which is isolated (Photo 5, Annex 1), therefore parameters are always higher than the rest of the system and are treated separately in this report; however, it was important to monitor this site, since there are plans to establish new tourist camps on its shores.

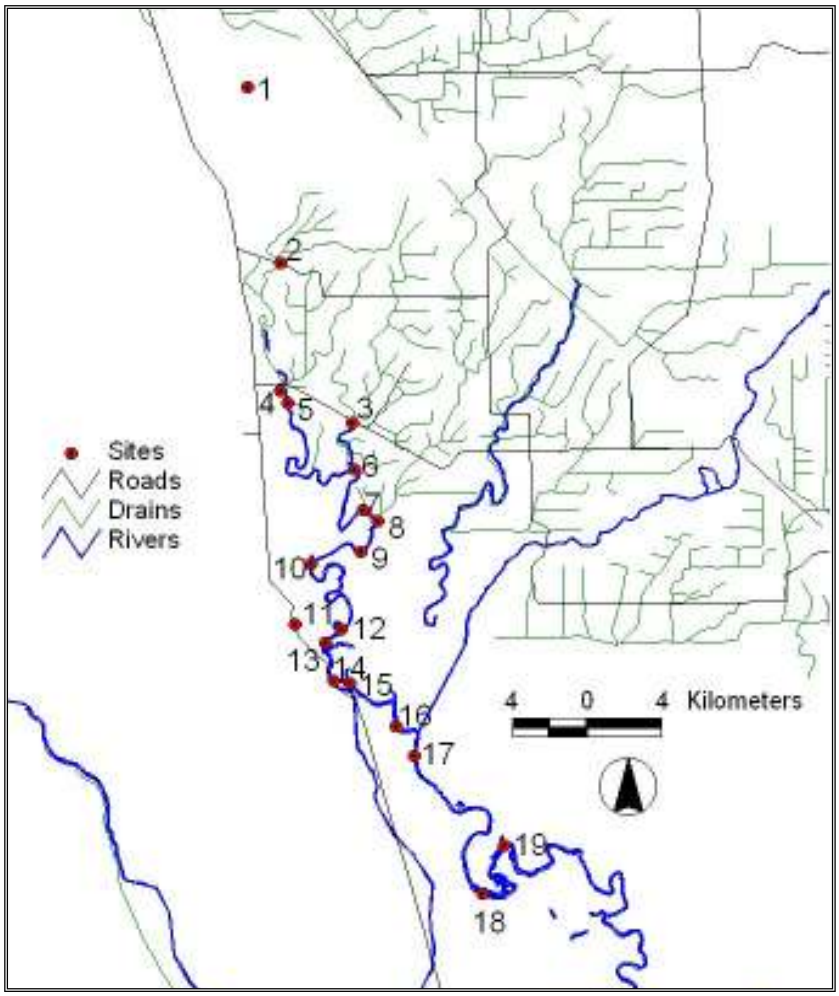

Fig. 1. Complete set of sampling sites on the Rio Hardy (2006 and 2008)

Table 1. Location, name and year of sampling of the 19 sites on Rio Hardy

No.	Site	UTM (X)	UTM (Y)	Year of sampling
1	Las Arenitas	657693	3587305	2008
2	Dren Nayarit	659466	3578107	2008
3	Dren Durango	663279	3569680	2008
4	Río Hardy 1	659458	3571370	2006
5	Río Hardy 2	659847	3570749	2006
6	Río Hardy 3	663349	3567210	2006
7	Río Hardy 4	663836	3565084	2006
8	Río Hardy 5	664628	3564555	2006
9	Río Hardy 6	663728	3562929	2006
10	Río Hardy 7	661077	3562297	2006
11	Campo Sonora	660197	3559115	2008

CONTINUATION…				
12	Campo Mosqueda	662659	3558848	2006 and 2008
13	Campo Las Cabañas	661847	3558122	2006
14	Campo El Mayor	662260	3556141	2006
15	Comunidad Cucapa	663109	3556022	2006 and 2008
16	Campo Flores	665554	3553736	2006
17	Campo Muñoz	666560	3552213	2006 and 2008
18	Campo Ramona	670137	3544988	2006 and 2008
19	El tapón	671313	3547448	2006

3.2. Microbiological parameters

Although the project started in February 2008, we did an exploratory survey at sites 1 and 2 in October and December 2007 and in January 2008, these surveys were made because the WTP started to operate and released effluents to the Hardy at the end of October. Results indicated extremely high counts of *E. coli*: On October 2007 at site 1, counts were 35,000 MPN/100 ml, on December 2007 counts were 686,700 MPN/100 ml and 488,400 MPN/100 ml at site 2, on January 2008, values decreased to 8,400 and 1,000 MPN/100 ml at sites 1 and 2 respectively (Fig. 1). Although sampling was not made on a 24 hour period as the NOM-001-ECOL-1996 indicates for effluents, these instant values do indicate that there was a problem with the treatment in the period between November 2007 and January 2008 effluents with dangerous contents of fecal matter, as *E. coli* indicates, were released into the Rio Hardy. This event caused serious environmental and social problems. Reports of bad odors, green coloration of waters and fish die-offs were published in local papers (www.lacronica.com) and Hardy users blamed CESPM (Comisión Estatal de Servicios Públicos de Mexicali) for not treating the water properly. This crisis resulted in the suspension of the WTP flows to the Rio Hardy at the beginning of February (Photo 2 and 3, Annex 1) by CESPM. Water remained in a pond at the WTP for three months (February to April 2008) in order to extend the residence time and comply with NOM-001-ECOL-1996 before entering the river.

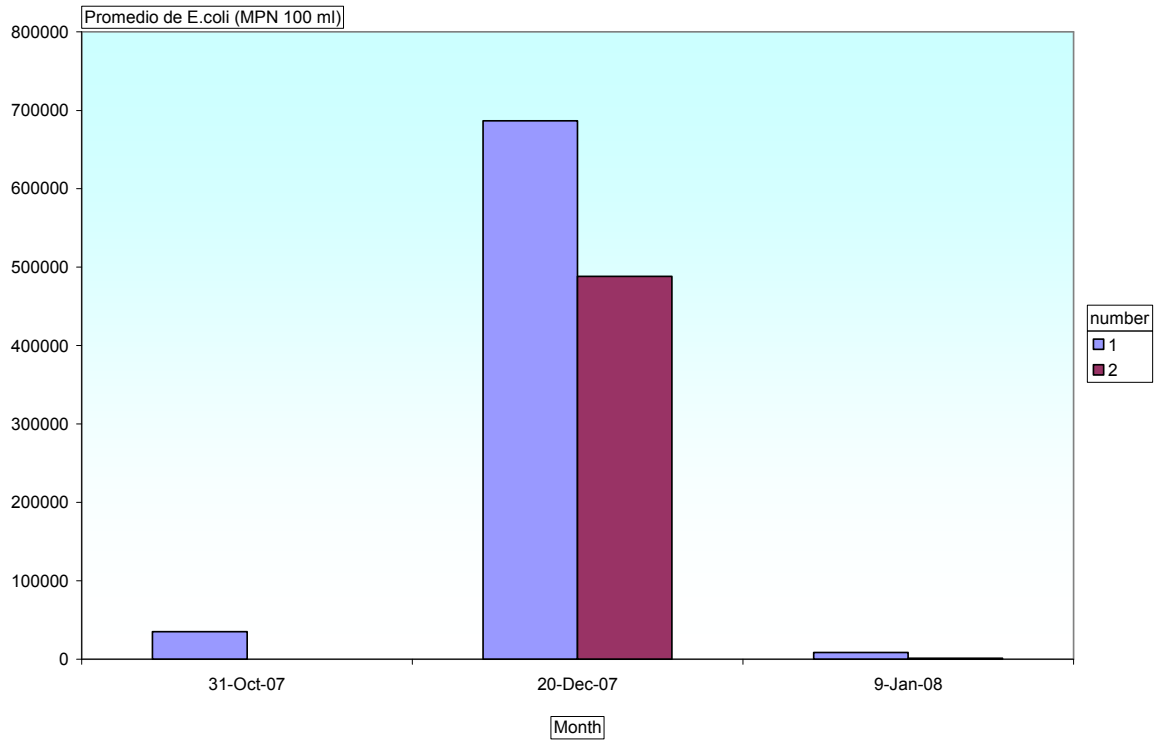

Fig. 2. *E. coli* concentrations at sites 1 and 2 on October, December 2007 and January 2008

The increase in residence time and other measures taken by CESMP, did lowered *E. coli* concentrations significantly at the effluent, and from February 2008 to February 2009, values at site 1 did not exceeded 46 MPN/100 ml (Fig. 3). At site 2, which is 10 km south from the plant, values were higher (max 308 MPN/100 ml in August) probably due to high summer temperatures and the presence of nutrients during the travel from the plant to the river (Fig 3).

On the Rio Hardy, concentrations of *E. coli* varied among sites. Site 11 (Campo Sonora) presented the higher values throughout the year (average 958 MPN/100 ml) (Fig. 4) because it is an isolated water body with high evaporation rates. We recommend restoring this water body before starting any tourist activity. Restoration should include the opening of inflows and outflows with Rio Hardy water, and a continuous monitoring of parameters.

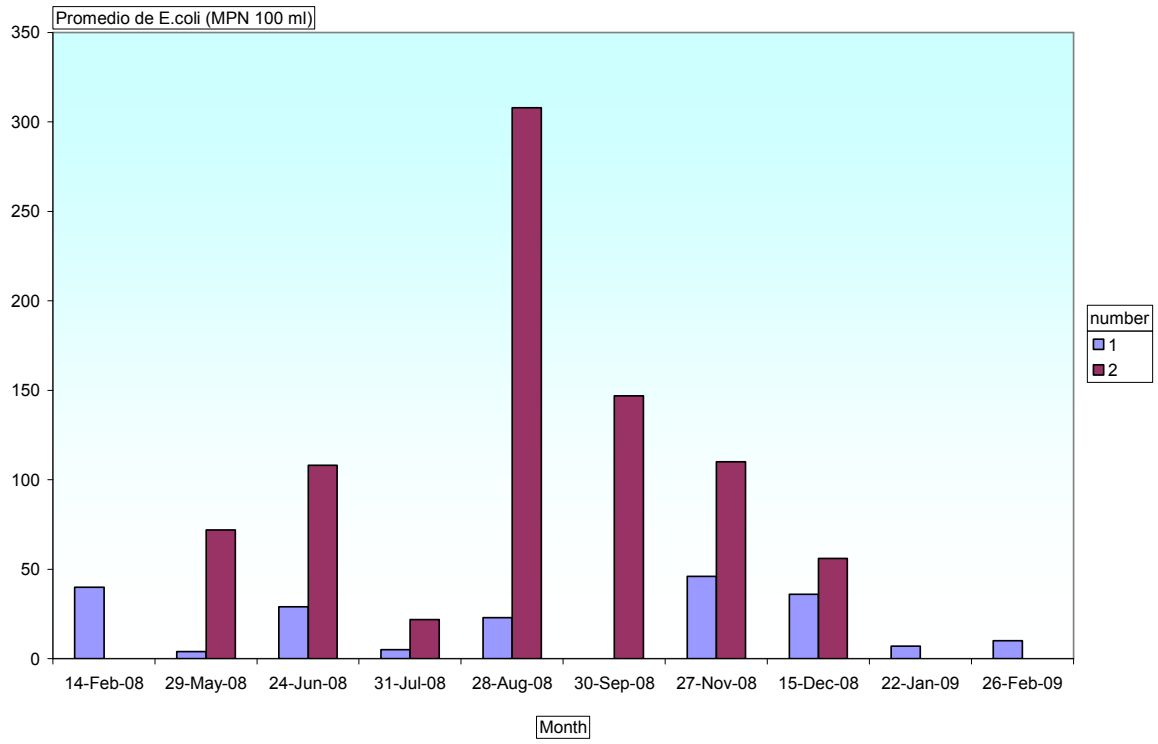

Fig. 3. *E. coli* concentrations at sites 1 and 2 from February 2008 to February 2009

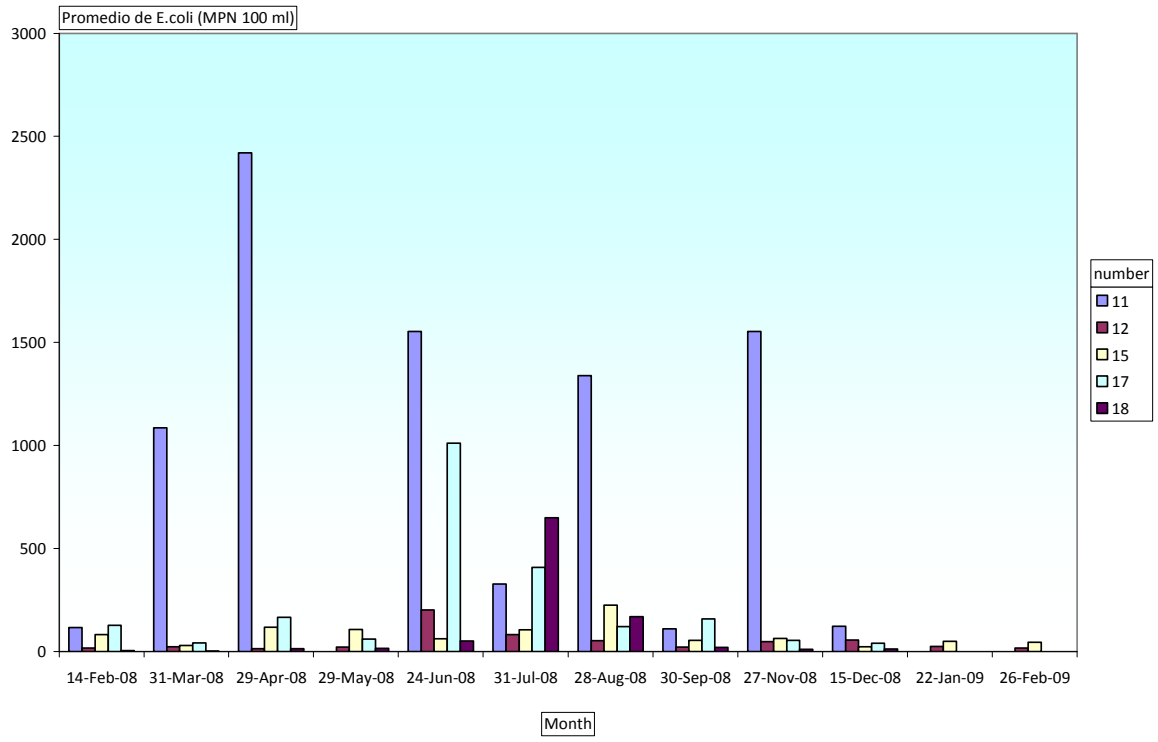

Fig. 4. Average concentrations of *E. coli* per month at 5 sites on the Rio Hardy, from February 2008 to February 2009.

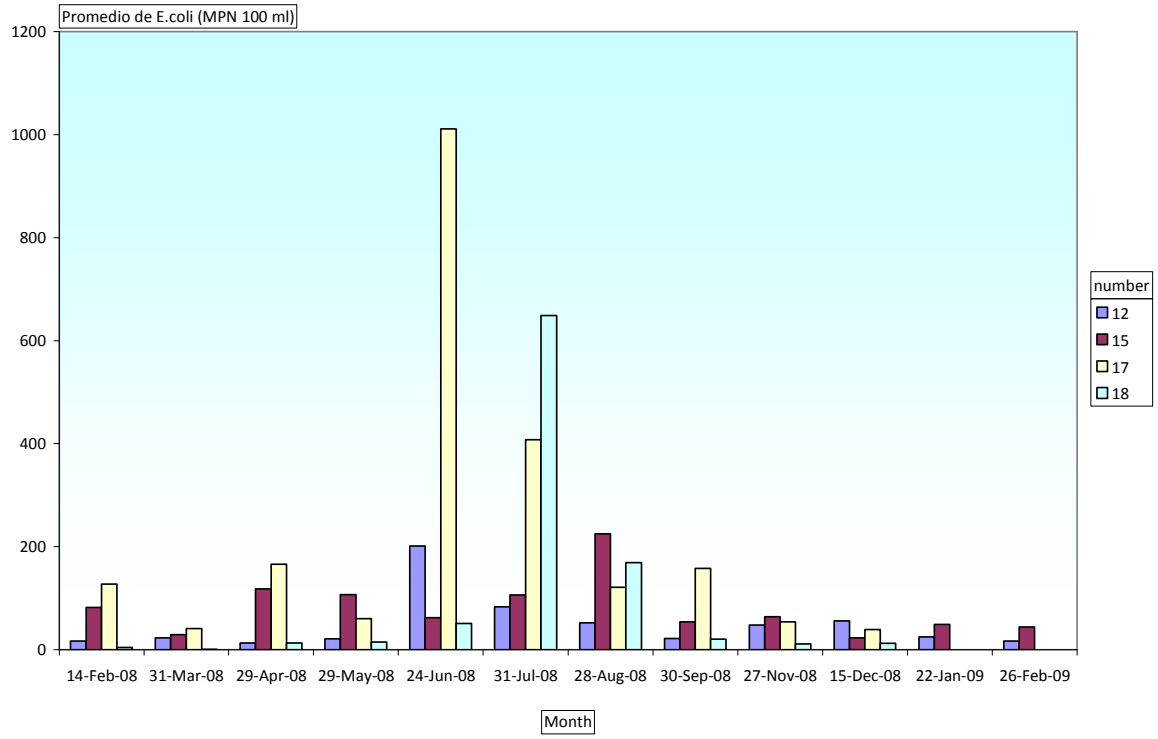

Fig. 5. *E. coli* concentrations per month at 4 sites (excluding site 11) from RH

According to the U.S. EPA *Implementation Guidance for Ambient Water Quality Criteria for Bacteria* (2002), the gastrointestinal illness rate (per 1000 people) for *E. coli* in fresh water is 8, the geometric mean density (GM) is 126 in 100 ml and the single sample maximum allowable density (SM) for a designated beach area is 235. California has set its water quality criteria for the Colorado River for primary recreational contact at 235 single sample maximum allowable density and for secondary recreational contact (where contact and immersion with the water are unlikely) at 1,175.

At site 11, *E. coli* concentrations are above the EPA criteria for primary and secondary recreational contact at least at one month per year, and since there is no apparent trend (i.e. high in summer low in winter), we recommend to avoid any recreational activity at this site all year until restoration measures are taken.

At site 12 and 15 (Mosqueda and Cucapa) *E. coli* concentrations in 2008 did not exceeded EPA criteria at any month sampled, therefore, all recreational activities are not limited by this parameter, although monitoring is recommended (Fig. 5)

Site 17 (Muñoz) exceeded criteria for primary contact on June and July and site 18 (Ramona) exceeded criteria on July (Fig. 5). It is possible that the Colorado River or local sources increased *E. coli* concentrations on the river at these two sites on June and July, because if increases were only due to high temperatures and evaporation, values from August and September would expected to be higher as well.

Comparing sites 12, 15, 17 and 18 between 2006 and 2008, we observe differences in the counts of *E. coli* concentrations (Fig. 6) which were confirmed by a t-test (*p-value* = 0.04), mean concentration prior the WTP was 2,195 MPN/100 ml and after it was 389 MPN/100 ml. These results suggest that water released from the plant from February 2008 to February 2009 has helped to lower *E. coli* concentrations at all stations along the Rio Hardy, and sites 12 and 15 have remained within EPA criteria.

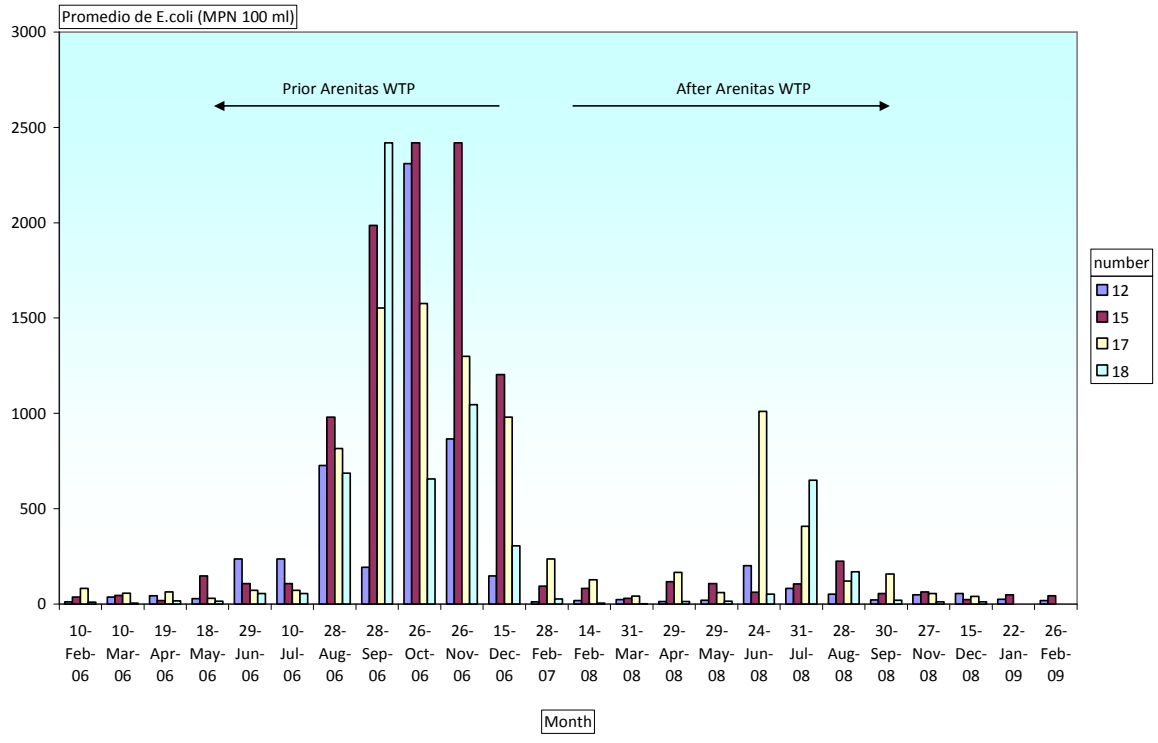

Fig. 6. *E. coli* concentrations at sites 12, 15, 17 and 18 from Feb 2006 to Feb 2007 and from Feb 2008 to Feb 2009 (Prior and after Arenitas WTP, respectively)

In February and April 2008, we did a test between the UABC and the CESPM laboratories. Although methods were different (we used Colilert kit and they used the traditional tube method), results were very similar (Table 2). We continued to use the kit and since it is specific for *E. coli*, we continue to analyze this parameter. CESPM has published water quality results from Rio Hardy and Arenitas WTP from September 2008 to March 2009 at their web page: (http://www.cespm.gob.mx/publicacionesresultado.php?claveTemaPDF=15). A summary is presented in Fig. 6. They sampled at the WTP effluent (site 1), at the WTP pond effluent (1.1), at puente Nayarit (2) and Campo Mosqueda (12). The highest values were reported from the Nayarit drain (2) in October and March, and from the pond effluent (1.1) on January 2009, but values at Mosqueda remained lower than the 235 criteria. It is possible that availability of nutrients increase coliform populations inside the lagoon and on the drains. It is very important that CESPM continues with this monitoring effort and its publication, since it helps local residents and users to understand river conditions, it is important, however, to interpret the data, and we offer to continue to do that through the local organizations like AEURHYC or Sonoran Institute.

Table 2. Test results from the UABC laboratory and CESPM laboratory

No.	Site name	Date	*E. Coli* analyzed at UABC NMP/100 mL	Fecal coliforms analyzed at UABC NMP/100mL	Fecal coliforms analyzed by CESPM NMP/100mL
3	Durango	19-Feb-08	31	43	43
3	Durango	31-Abr-08	300	430	240

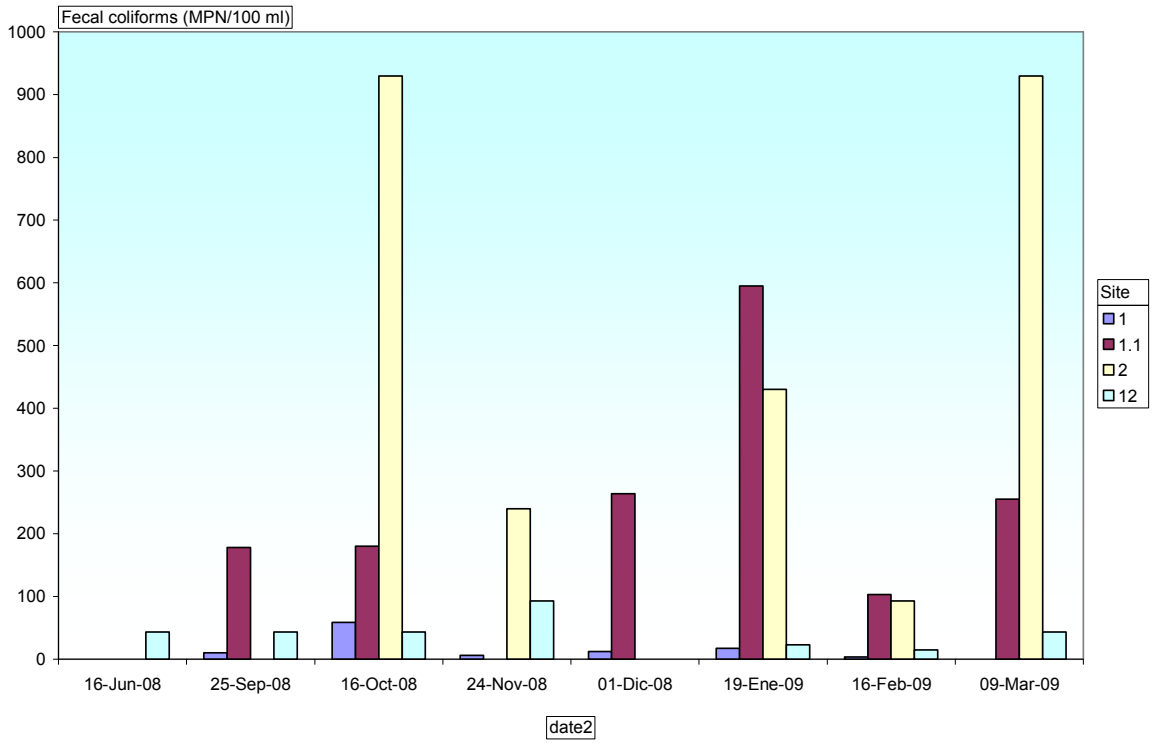

Fig. 6. Fecal coliforms measured by CESPM at site 1, 2 and 12 in 2009.

On May 2008, samples were analyzed by a private laboratory in Mexicali (Laboroatorios Mayo) in order to measure other microorganisms commonly found in the area, like Salmonella and free living amoeba. Results indicated that fecal coliforms were under detection limit (< DL), Salmonella was absent and free living amoeba (FLA) was also absent, they identified other microorganisms commonly found in the environment (Table 3).

Table 3. Concentrations of microorganisms in water collected at Las Arenitas and two sites in the Rio Hardy on May 29th, 2008 (data analyzed at a private laboratory).

Site No.	Aerobic mesophilic UFC/mL	Total coliforms MPN/100mL	Fecal coliforms MPN/100	Salmonella	Free living amoeba (FLA)	Identified microorganism
1	2,000	240	< DL	Absent	Absent	*Proteus vulgaris* and *Enterobacter agglomerans*
2	13,000	210	< DL	Absent	Absent	*Proteus vulgaris* and *Citrobacter diversus*
2	16,000	240	< DL	Absent	Absent	*Proteus vulgaris* y *Citrobacter diversus*
12	1,200	43	< DL	Absent	Absent	*Proteus vulgaris*

3.3. Nutrients

Nutrients that are of great concern in riparian and estuarine ecosystems are nitrogen and phosphorus. Their sources typically are discharges from sewage treatment plants and industries and runoff from urban and agricultural areas (U.S. EPA, 1994). Increased nutrient levels lead to phytoplankton blooms and a subsequent reduction in DO levels and light penetrations. The term eutrophication is defined as the process of nutrient enrichment in a water body and is also used to describe the effects of nutrient enrichment. Eutrophication is greatly accelerated by anthropogenic nutrient enrichment (US EPA, 1994).

We measured nitrates (NO_3) in 2006, 2007 (3 months) and 2008 at sites 12, 15, 17, 18 on the river (Fig. 7). A peak on February 2008 was evident, as a result of the WTP operations, however, we did not observed a significant difference prior and after the operation of the plant (t-test, *p-value* = 0.15).

Nutrient standards for Arizona are presented in Table 4. The NOM-001-ECOL 1996, allows 60 mg/l of nitrates at discharges that will be use for agricultural use.

Mean annual concentrations of nitrates were lower than the NOM-001 guideline at Arenitas, however they were higher than the US EPA guideline of 5 mg/l at site 1 (Arenitas). Also, the months of November, December 2008 and February 2009 nitrates exceeded the guideline at sites 2 and 3 (Fig. 8). However, on the river and excluding February 2008, which

was sampled during the adjustments of the plant, and site 11 (Campo Sonora) from the analysis, no concentrations were higher than 5 mg/l in the period sampled.

It is interesting to note that high concentrations at the WTP effluent and upstream drains did not increased values along the river, at least not at the sites sampled (from Mosqueda south). It is possible that algae are taking the extra nutrients from the water column resulting in low measurable levels downstream. On August, when values were higher than 4 mg/l at the discharge, concentrations downstream decreased considerably and this trend continued the following months. Algae growth is a symptom of eutrophication and can cause oxygen depletion at the river and possible fish die-offs.

Table 4. Arizona Water quality standards (US EPA, 2000)

"*The mean annual total phosphate and mean annual total nitrate concentrations of the following waters shall not exceed the values given below nor shall the total phosphate or total nitrate concentration of more than 10 percent of the samples in any year exceed the 90 percent values given below. Unless otherwise specified, indicated values also apply to tributaries to the named waters*"

	Mean 90 pct annual value	
Area	Total phosphates as PO_4 mg/l	Total nitrates as NO_3 mg/l
Colorado River from Imperial Dam to Morelos Dam (main stem)	0.10-0.10	5-7

"*The above standardards are intended to protect the beneficial uses of the named waters…*"

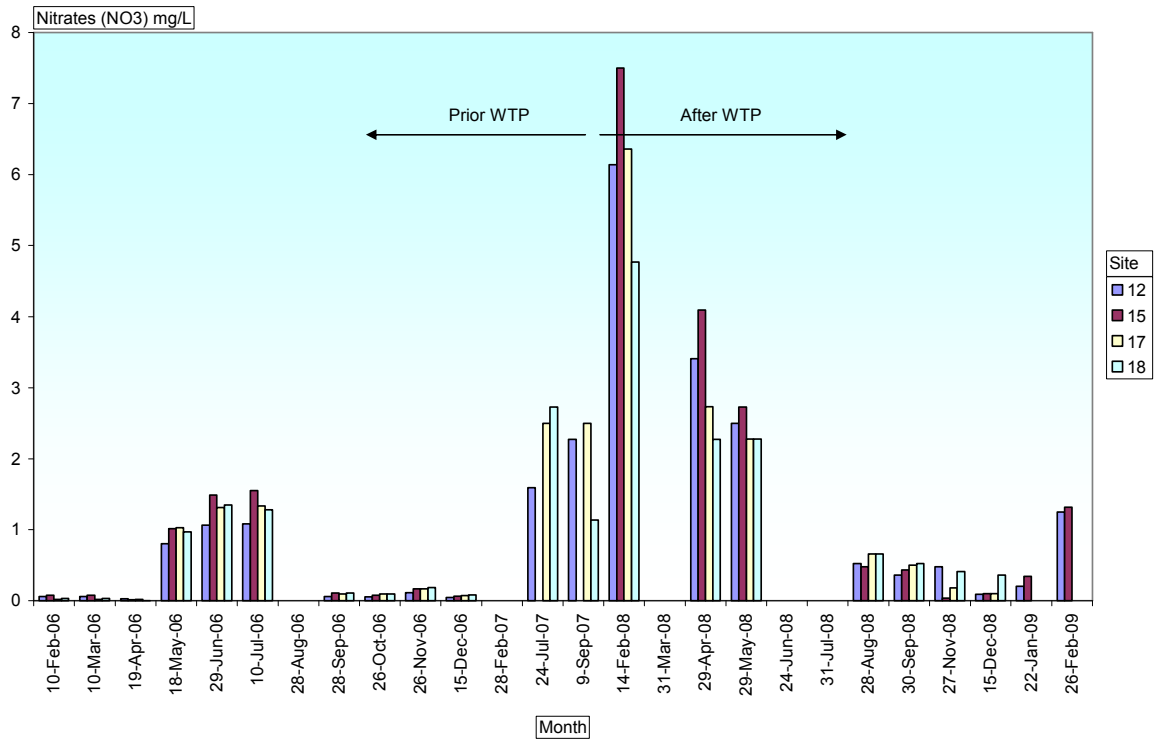

Fig. 7. Nitrate (mg/l) concentrations at sites 12, 15, 17 and 18 prior and after Arenitas WTP.

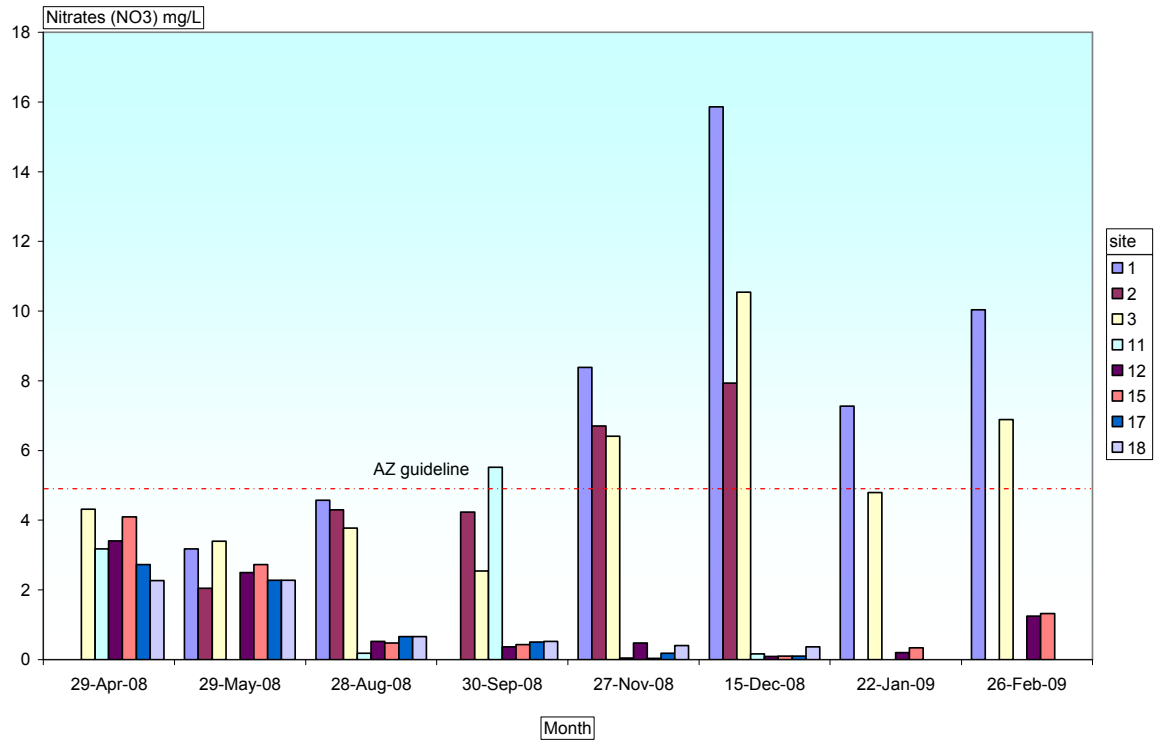

Fig.8. Nitrates (mg/l) after the WTP operation at effluent and river sites

After oxygen, ammonia is the most important water quality parameter that affects fish. It causes stress, damages gills and tissues even in small amounts. Fish exposed to low levels of ammonia over time are more susceptible to bacterial infections and have poor growth. In aqueous ammonia solutions, un-ionized ammonia exists in equilibrium with the ammonium ion and the hydroxide ion. The dissolved un-ionized ammonia is represented as NH_3. The ionized form is represented as NH_4^+. The term total ammonia refers to the sum of these. The toxicity of aqueous ammonia solutions to aquatic organisms is primarily attributable to the NH_3 species, with the NH_4^+ species being relatively less toxic. Un-ionized ammonia is dependent to pH and temperature, the concentration of NH_3 increases with increasing pH and with increasing temperature, using total ammonia concentrations, un-ionized ammonia can be calculated with a table produced by Emerson et al 1975 (US EPA, 1985).

At Rio Hardy, un-ionized ammonia concentrations exceeded mean fish toxicity limits (LC_{50}) for striped mullet (*Mugil cephalus*), channel catfish (*Ictalurus punctatus*), green sunfish (*Lepomis cyanellus*) and blue gill (*Lepomis machrochirus*) at sites 1, 2 and 3 after the operation of the WTP. However, from site 12 and south un-ionized concentrations did not exceeded the limit, with the exception of site 11 that exceeded the limit on September (Fig. 9). Therefore, it is likely that high un-unionized ammonia levels, together with low oxygen concentrations (due to algal growth), at the Hardy north could be the reasons for recurrent fish die-offs reported on this area by local residents.

Prior to the operation of the WTP phosphates were under the US EPA guideline for Arizona, however, beginning in February 2008, values increased considerably and there were peaks in August, November 2008 and February 2009 all above AZ guideline (Fig. 10). Sewage treatment plants are typically the major source of nutrients, particularly phosphorus, to estuaries in urban areas (US EPA 1994).

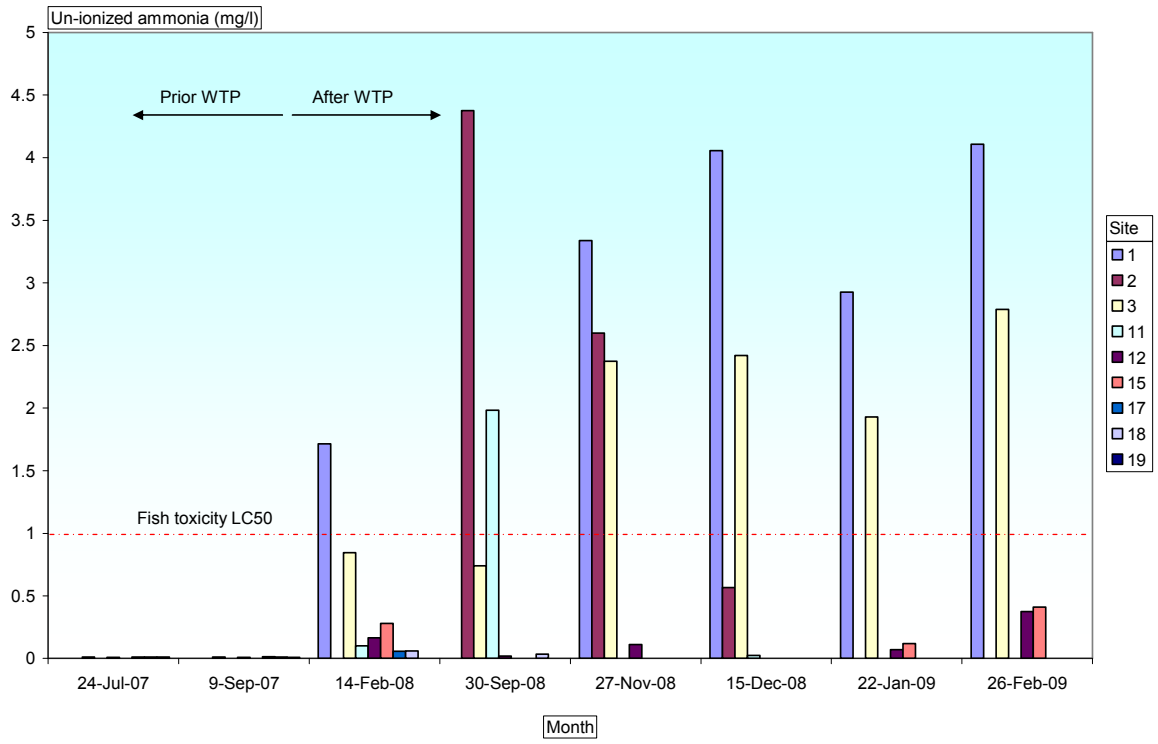

Fig. 9. Concentration of un-ionized ammonia (mg/l) prior and after the WTP at the inflows and Rio Hardy.

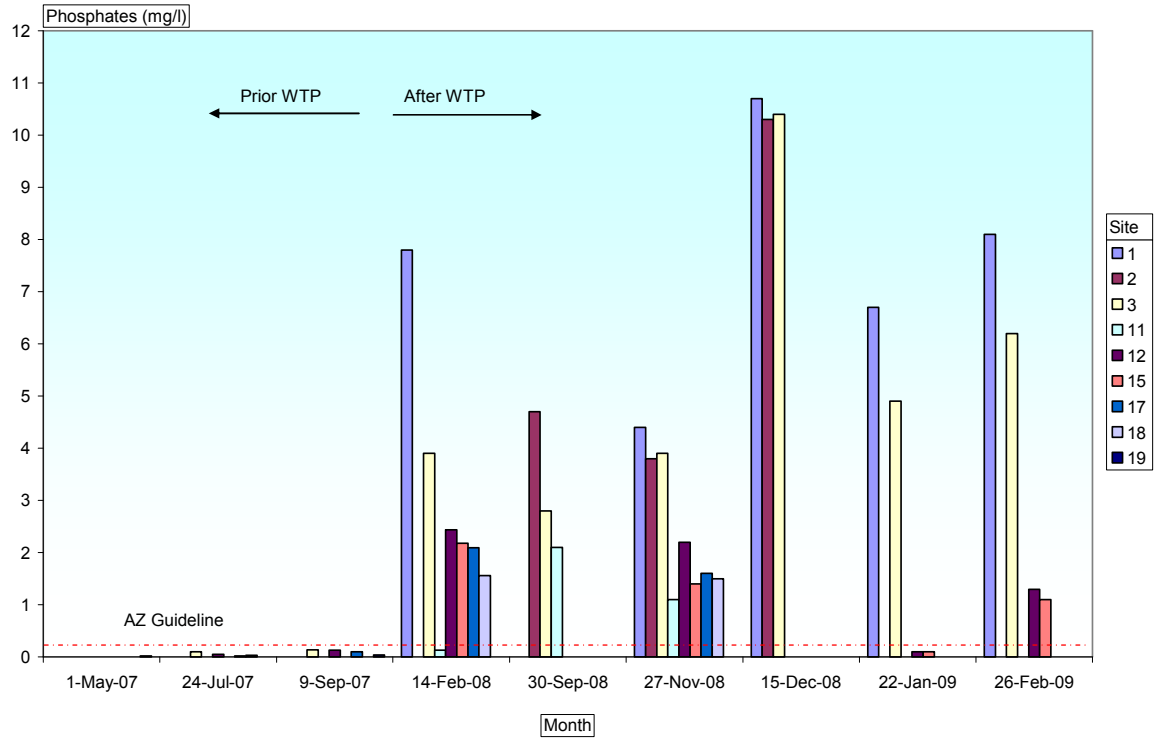

Fig. 10. Phosphate concentrations (mg/l) prior and after the WTP at the inflows and Hardy

3.4. Field parameters

One of the most important parameter for restoration projects is salinity (reported here as total dissolved solids, TDS). At sites 12, 15, 17 and 18, mean TDS values prior the operation of the WTP were 6,469 ppm, and after the WTP mean concentrations lowered to 4,416 ppm (Fig. 11). At Mosqueda, site 12, mean TDS decreased from 5,281 ppm to 4,025, at Cucapa, it decreased from 6,200 to 4,349 ppm, at Muñoz, it went from 6,700 to 4,846 ppm and at Ramona, concentrations lowered from 7,478 to 5,300 ppm. Also, the standard variation between months was lower after the WTP (1,533) than prior (1,961), i.e. differences between winter and summer months were less marked after the WTP. Although these are monthly measurements, they do indicate a trend that will need to be confirmed with conductivity loggers that will measure values on an hourly basis and that can be installed at these four sites.

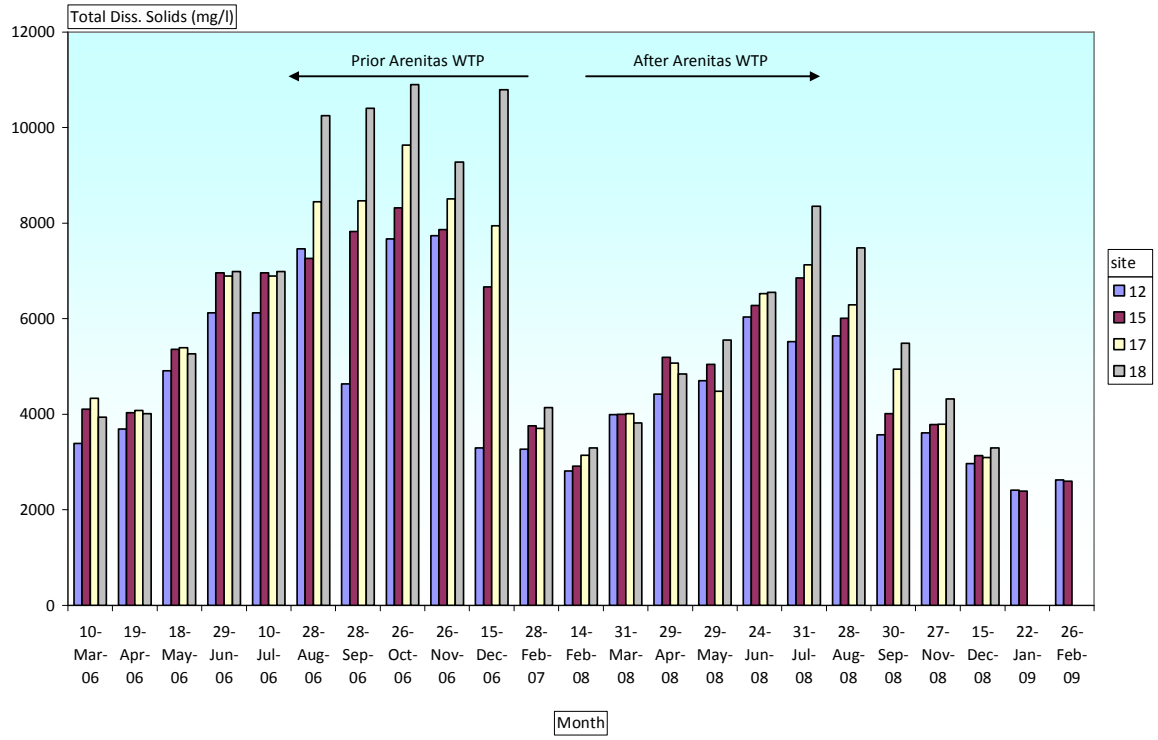

Fig. 11. TDS (mg/l) prior and after Arenitas WTP at four sites in the Rio Hardy

At these five sites, mean temperature did not varied prior and after the WTP (*p-value* = 0.97). However, pH has increased after the WTP releases from 8.2 to 8.4, although not significantly (*p-value* = 0.07).

Dissolved oxygen varies greatly between day and night, in our field measurements we never detected anoxia and few were under 3 mg/l, however, there was a significant difference between mean values prior and after the WTP. Higher concentrations were measured after the WTP discharges (mean = 10.7 mg/l) than prior (mean = 6.03) (*p-value* = 0.01). Although not all measurements were made at the same hour of the day, they do indicate a trend that needs to be confirmed using data loggers at some sites like Mosqueda and Ramona. It is likely that nutrients released from the WTP stimulate algae growth that generate greater quantity of oxygen during the day but also take oxygen during the night, causing anoxia and possible fish die-offs.

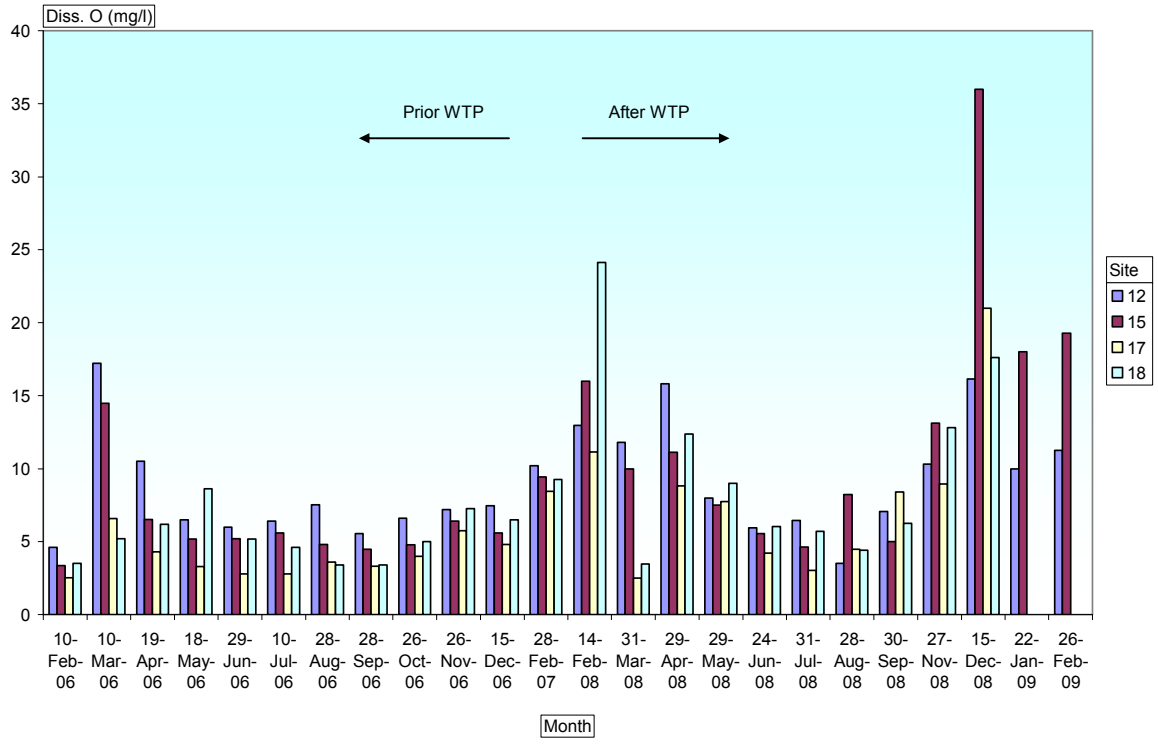

Fig. 12. Oxygen concentrations prior and after the WTP at four sites in the Rio Hardy

3.5. Toxicants

The Rio Hardy is exposed to a variety of toxic chemicals derived from the agricultural activities, from natural sources, and from sewage and water treatment plants. It is important to measure traces of heavy metals and organic compounds in different matrices. However, not all chemicals can be measured nor the effect of mixtures can be assessed, this is why it is also important to evaluate the effects chemicals are having on the resident fauna. In addition to trace analysis, we present results on the effects of organophosphate pesticides in Rio Hardy barnacles measured as the percent inhibition of the acetilcholinesterase (AchE) enzyme.

On February 14, 2008, after four months of the operation of Arenitas WTP, we collected water and sediment samples at Nayarit (# 2) and Durango (# 3) drains and at Campo Sonora (11), Mosqueda (12), Cucapa (15), Muñoz (17) and Ramona (18), and fish at the confluence of Durango drain and Rio Hardy and south at Ramona (Table 5).

Table 5. Type of matrix collected at each site on Feb 14, 2008 at the Rio Hardy

Site No.	Water	Sediment	Fish
2	1 grab sample	1 composite sample	No fish sampled
3	1 grab sample	1 composite sample	5 Carps (*Cyprinus carpio*), 1 largemouth bass (*Mycropterus salmoides*)
11	1 grab sample	1 composite sample	No fish sampled
12	1 grab sample	1 composite sample	No fish sampled
15	1 grab sample	1 composite sample	No fish sampled
17	1 grab sample	1 composite sample	No fish sampled
18	1 grab sample	1 composite sample	2 Mullets (*Mugil cephalus*)

Concentrations of metals in water were all lower than the NOM-001-ECOL-1996 for agricultural and ecological use (Table 6). In sediments, Pb and Hg exceeded the Lowest Effect Level (LOEL) for protection of benthic organisms (Table 7), and in fish tissue, concentrations of cadmium exceeded the limit set by the NOM-027-SSA for protection of human health. Also, one sample exceeded the SSA limit for mercury, this was the largest carp collected (1.4 kg), and is likely that larger, hence older individuals, accumulate higher concentrations of mercury.

Table 6. Concentrations of metals in water from Rio Hardy collected on Feb 2008

Site	Cd (mg/l)	Pb (mg/l)	Hg (mg/l)
2	0.024	0.00	0.00096
3	0.038	0.00	0.00255
11	0.079	0.11	0.00006
12	0.031	0.00	0.00304
14	0.046	0.00	0.00011
17	0.042	0.00	0.00144
18	0.060	0.00	0.00134
NOM-001[1]	(0.40, 0.20)	(1.00, 0.40)	(0.02, 0.01)
CSC[2]	0.057	0.00	0.00100

[1]NOM-001-1996-ECOL (agriculture use, protection of aquatic life)
[2]Cienega de Santa Clara

Table 7. Concentrations of metals in sediment from Rio Hardy on Feb 2008

Site	Cd (mg/l)	Pb (mg/l)	Hg (mg/l)
2	5.344	57.252	0.1293
3	8.412	78.247	0.1738
11	8.668	59.102	0.2720
12	7.672	38.358	0.0383
14	7.674	78.709	0.3049
17	4.675	19.478	0.1073
18	8.234	38.300	0.0382
LOEL[1]	10.00	31.00	0.20
cienega	8.333	56.818	0.0751

[1]Lowest Effect Level (Persaud D. et al, 1993)

Table 8. Concentrations of metals in fish (whole body) from Rio Hardy on Feb 2008

Species	Total lenght (cm)	Weight (g)	Cd (mg/l dry wt)	Pb (mg/l dry wt)	Hg (mg/l wet wt)
Mugil cephalus	29.3	271.1	1.371	19.592	0.050
Mugil cephalus	31	287.1	3.808	38.081	0.000
Cyprinus carpio	22.5	167.5	0.770	38.521	0.011
Cyprinus carpio	19	120.6	1.147	19.113	0.016
Mycropterus salmoides	19.5	113.8	2.265	18.875	0.000
Cyprinus carpio	35	646.9	0.777	19.417	0.000
Cyprinus carpio	44	1171.2	1.547	38.685	0.016
Cyprinus carpio	47	1460.9	0.994	39.746	1.850
NOM-027-SSA			0.50	1.0	1.0

Table 9. Concentrations of metals in barnacles collected during 2007 in Rio Hardy

Site	Cd (mg/kg)	Pb (mg/kg)	Hg (mg/kg)
3	0.125	0.188	0.002
12	0.221	0.063	
17	0.105	0.100	0.058
18	0.190	0.042	0.066
19	0.185		0.043
19 south	0.178	0.125	0.010

Figs 13 to 15 show concentrations of metals in sampling periods prior and four months after the operation of the plant, we did not observed increases in metal concentrations in any type of matrix: water, sediment or fish. However, new samples are needed after a larger period of operation time, in order to draw any conclusion about this subject.

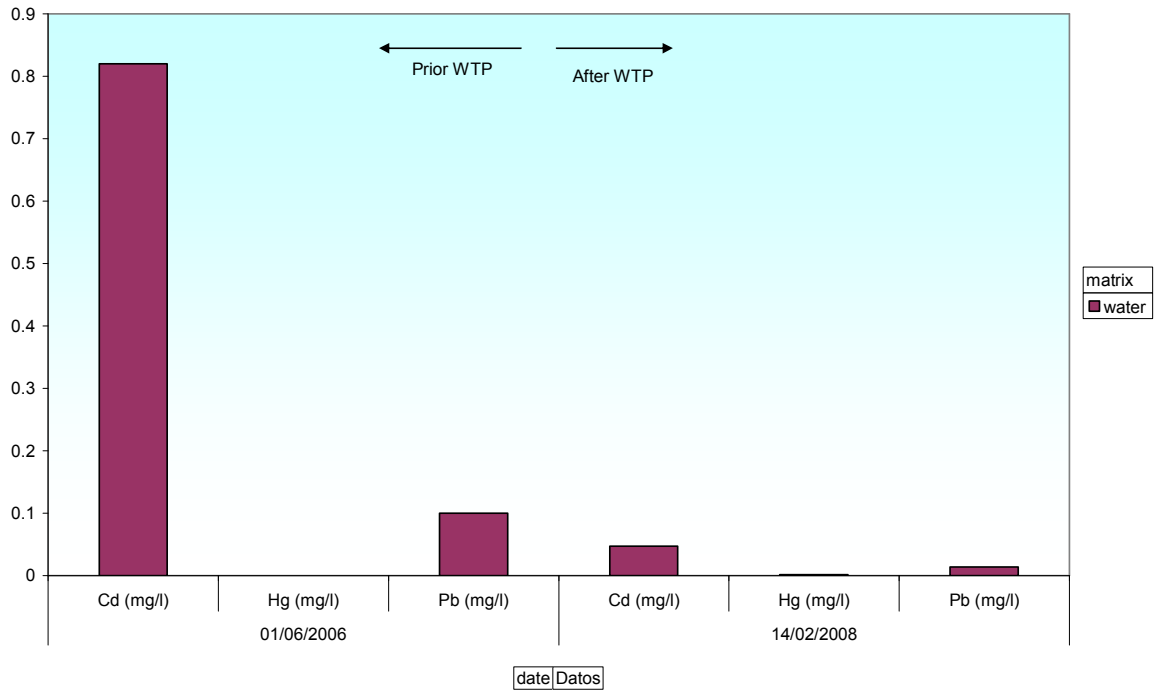

Fig. 13. Concentrations of metals in water prior and after the operation of the plant

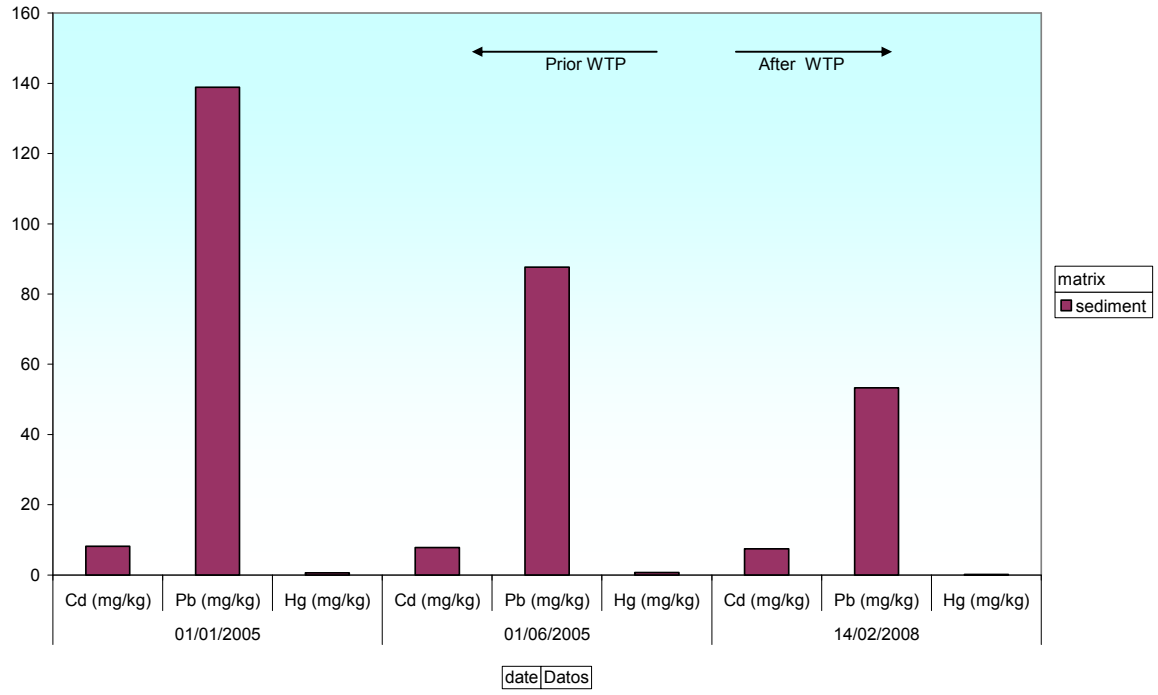

Fig. 14. Concentrations of metals in sediment prior and after the operation of the plant

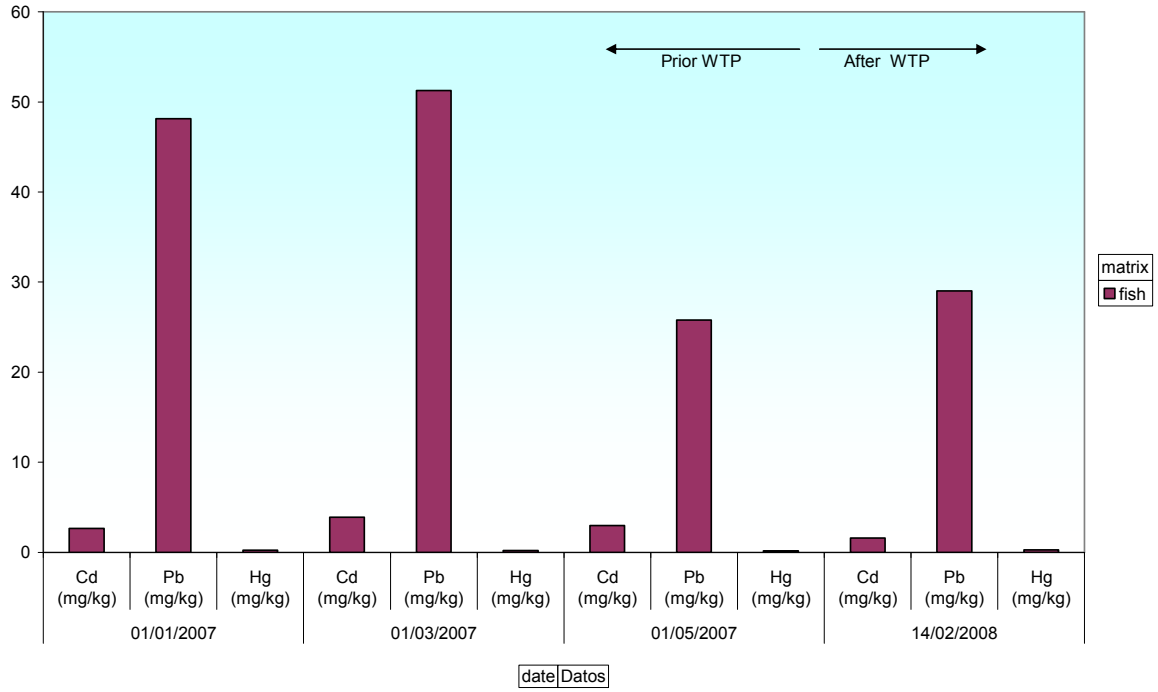

Fig. 15. Concentrations of metals in fish tissue prior and after the operation of the plant

Other toxicants are organic compounds, we have analyzed organochlorine concentrations in fish from Rio Hardy (Table 10). The NOM-027-SSA prohibits the presence of any pesticide in fish, we detected a mean of 0.01 ppm of Aldrin and a mean of 0.06 ppm of DDE in fish. DDE is of great concern, since values exceeding 0.01 ppm could affect the health of local residents who consume fish from the Hardy often (US EPA, 2000b).

Table 10. Concentrations of organochlorine pesticides (µg/g, wet wt) in fish from the Hardy River collected on February 2008.

Common name	**Weight (g)**	**Total length**	**Site No.**	**BHC**	**Lindane**	**Heptachlor**	**Aldrin**	**Endrin**	**Metoxichlor**	**DDE**	**DDD**	**DDT**
Guideline							5.65 *			0.0144 **		
Mullet	271	29	7	< DL	< DL	< DL	0.0025± 0.0001	< DL	< DL	0.0433 ± 0.0030	< DL	< DL
Mullet	287	31	7	< DL	< DL	< DL	0.0212 ± 0.0049	< DL	< DL	0.0981 ± 0.0005	< DL	< DL
Carp	168	23	2	< DL	< DL	< DL	0.0114 ± 0.0008	< DL	< DL	0.0392 ± 0.0025	< DL	< DL
Carp	121	19	2	< DL	< DL	< DL	0.0114 ± 0.0017	< DL	< DL	0.0388 ± 0.0053	< DL	< DL
Carp	647	35	2	< DL	< DL	< DL	0.0023 ± 0.0008	< DL	< DL	0.1000 ± 0.0023	< DL	< DL
Carp	1171	44	2	< DL	< DL	< DL	0.0290 ± 0.0032	< DL	< DL	0.0775 ± 0.0087	< DL	< DL
Carp	1461	47	2	< DL	< DL	< DL	0.0174 ± 0.0021	< DL	< DL	0.0544 ± 0.0064	< DL	< DL
Largemouth bass	114	20	2	< DL	< DL	< DL	0.0043 ± 0.0004	< DL	< DL	0.0509 ± 0.0032	< DL	< DL
Mean							**0.0124 ± 0.0017**			**0.0628 ± 0.0040**		

*Shubat. 1998. (Fish survival -reduced > 50%).
**Screening values for tDDT for cancer health effects for subsistence anglers (i.e. native Cucapa and local residents) based on a daily consumption rate of 142.2 g of fish, 70 kg body weight and 1 in 100,000 risk level and 70 yr lifetime (US EPA 2000b).

In sediments, no residue of organochlorine compounds was detected, however Polyciclic Aromatic Hydrocarbons were detected. Previous samplings in the Rio Hardy in 2005 showed a range of DDE concentrations from 0.003 to 0.08 ppm with a mean of 0.03 ppm (Ramos, 2008).

Table 11. Concentrations of organochlorine pesticides (µg/g) in sediments from the Hardy River collected on February 2008.

Site No.	BHC	Lindane	Heptachlor	Aldrin	Endrin	Metoxichlor	DDE	DDD	DDT	PAHs*
1	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	Detected
2	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	Detected
3	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	Detected
4	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	Detected
5	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	Detected
6	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	Detected
7	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	Detected
8	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	Detected

*Using GC-Mass spectrophotometer, we detected Polyclic Aromatic Hidrocarbons (PAHs) in all samples

3.6. Biomarkers

Table 12. Acetilcholinesterase inhibition (%) in barnacles from the Rio Hardy

Site No.	JAN 07	MAR 07	MAY 07	JUL 07	SEP 07	NOV 07	Mean
3	54.7	91.8	92.6		86.1		81.3
12	79.3		89.8		37		68.7
17	52.1	0	15	0	59.2		25.2
18	51.6		73	16.6	93.7		58.7
19		10.34		56.4	77.8		48.1
19 south		40	57.9	63.8	46	68.4	55.2

Table 13. AChE inhibition in barnacles from a reference site (Estero del Soldado)

Conc of OP mixture	% inhibition
0 ppb (control)	0
2 ppb	43.5
5 ppb	33.1
10 ppb	31.5

Currently, the most commonly used pesticides in the Mexicali valley are Organophosphates (OP), these compounds are very toxic but they are easily degraded in the environment and are difficult to detect in water, sediment or fish. However, their effects can be measured by testing the percentage of Acetilcholineserase (AchE) inhibition in organisms, preferably

sessile organisms from the ecosystems. In our case, we use barnacles as test organisms, since they are present along the Rio Hardy and they are abundant. A reference sample was collected in an estuary in Guaymas, where no pesticides are present. Results indicated that organisms from the reference site presented 0% inhibition, however, if exposed to a mixture of OP pesticides in the laboratory they presented inhibition at the 2 ppb level of 43% (Table 11), after this concentration, it is likely that barnacles began to die and inhibition decreased due to organism failure.

In the Rio Hardy, mean percent inhibitions ranged from 25 to 81% (Table 12), this result indicates the presence of OP pesticides at concentrations higher than 2 ppb and possibly higher resistance of the organisms, since 81% inhibitions were observed. There were differences between sites. Inhibition was higher in barnacles from site 3, which is the major agricultural drain that feeds the Rio Hardy, inhibition averaged 81% . Barnacles collected from Campo Mosqueda (site 12) presented an average inhibition of 68% with higher values in May. At Campo Muñoz (site 17) where the Rio Colorado joins the Hardy, there was an average of 42% with higher inhibitions in September. At Campo Ramona (site 18), average inhibition is of 58% with higher inhibitions also in September. At site 19, we collected barnacles inside the dike (north) and outside (south), average inhibition at site 19 north were 48% with higher inhibitions in September. At 19 south, mean inhibition was 55% with higher inhibition in July.

4. CONCLUSIONS

4.1. *E. coli* concentrations at the upper Rio Hardy exceeded EPA criteria for recreational contact from October 2007 to January 2008; this was the period when Las Arenitas WTP started operations.

4.2. After measures were taken at the plant, concentrations of *E. coli* decreased significantly to levels within limits for recreational contact at sites 12 and 15 (Mosqueda and Cucapa) from February 2008 to our last sampling date in February 2009

4.3. Water released from the plant after February 2008, has significantly lowered *E. coli* concentrations at all stations along Rio Hardy.

4.2. Nitrate concentrations increased at the beginning of the operation of the plant in February 2008, however, values decreased afterwards, and they remained lower than US EPA guideline.

4.3. Higher concentrations at the effluents did not increased values downstream. It is possible that algae growth is taking the extra nutrients downstream. Algae growth is a symptom of eutrophication and can cause oxygen depletion and possible fish die-offs.

4.4. At Rio Hardy, un-ionized ammonia concentrations exceeded mean fish toxicity limits (LC_{50}) for striped mullet (*Mugil cephalus*), channel catfish (*Ictalurus punctatus*), green sunfish (*Lepomis cyanellus*) and blue gill (*Lepomis machrochirus*) at sites 1, 2 and 3 after the operation of the WTP.

4.5. It is likely that high un-unionized ammonia levels, together with low oxygen concentrations (due to algal growth), at the Hardy north could be the reasons for recurrent fish die-offs reported on this area by local residents.

4.6. Prior to the operation of the WTP phosphates were under the US EPA guideline for Arizona, however, beginning in February 2008, values increased considerably and there were peaks in August, November 2008 and February 2009 all above guideline.

4.7. At sites 12, 15, 17 and 18, mean total dissolved solids (TDS) values prior the operation of the WTP were 6,469 ppm, and after the WTP mean concentrations lowered to 4,416 ppm, also differences between winter and summer months were less marked

after the WTP. Lower concentrations of salts will definitely help in any restoration activities along the Rio Hardy, including the southern sites, like Campo Ramona (18).

4.8. Higher concentrations of dissolved oxygen were measured after the WTP discharges (mean = 10.7 mg/l) than prior (mean = 6.03). It is likely that nutrients released from the WTP stimulate algae growth that generate greater quantity of oxygen during the day but also take up water oxygen during the night.

4.9. Concentrations of metals in water were all lower than the NOM-001-ECOL-1996 for agricultural and ecological use (Table 6). In sediments, Pb and Hg exceeded the Lowest Effect Level (LOEL) for protection of benthic organisms, and in fish tissue, concentrations of cadmium, lead and mercury exceeded the limit set by the NOM-027-SSA for protection of human health.

4.10. No increases in metal concentrations were found after the WTP in any type of matrix: water, sediment or fish. However, new sampling is necessary, after more than a year of operation, in order to draw any conclusion about metal concentrations.

4.11. DDE was found in fish tissues above 0.01 ppm which could affect the health of local residents who consume fish from the Rio Hardy on a daily basis. This concentration was found prior the operation of the plant and is related with remnant levels of pesticides in soil and sediments from the Mexicali valley.

4.12. In the Rio Hardy, mean percent AchE inhibitions in barnacles ranged from 25 to 81% with a mean of 56% this result indicates the presence of OP pesticides at concentrations higher than 2 ppb and effects at the cellular level in resident organisms due to pesticides.

5.0. RECOMMENDATIONS

5.1. To continue the discharge of treated water from Las Arenitas WTP to Rio Hardy, at a flow of at least 0.55 cms in order to have an effect along al the Rio Hardy.

5.2. It is necessary to approve the project for the Constructed Wetland at Las Arenitas WTP. Aquatic plants are excellent filters and will decrease nutrient loads from the plant improving conditions along the Rio Hardy. If this project is not concluded, it is likely that fish die-offs continue on the river due to high nutrient loads from the plant.

5.3. To continue the monitoring of parameters by CESPM at various points along Rio Hardy and at Las Arenitas and continue with their publication in CESPM web page.

5.4. To install conductivity loggers at sites 12, 15, 17 and 18 (Mosqueda, Cucapa, Muñoz and Ramona) to monitor salinities and to install oxygen/nutrient/chlorophyll loggers at sites 12 and 18 to monitor algal growth.

6.0. ACKNOWLEDGMENTS

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7.0 REFERENCES:

NOM-001-ECOL. 1996. Norma Oficial Mexicana que establece los limites máximos permisibles de contaminantes en las descargas residuales en aguas y bienes nacionales. Publicada en el Diario Oficial de la Federación de fecha 6 de enero de 1997.

NOM-027-SSA. 1993. Bienes y servicios. Productos de la pesca. Pescados frescos-refrigerados y congelados. Especificaciones sanitarias.

Persaud D, Jaagumagi R, Hayton A. 1993. Guidelines for the protection and management of aquatic sediment quality in Ontario. Ontario Ministry of Environment and Energy, p. 24.

Shubat PJ, Curtis LR. 1986. Ration and toxicant preexposure influence of dieldrin accumulation by rainbow trout (*Salmo gairdneri*). Environ Toxicol Chem 5:69-77. In: Jarvinen AW and Ankley GT. 1998. Linkage of effects to tissue residues. SETAC Press.

Sykes, G. 1937. The Colorado Delta. Baltimore, Carnegie Institution of Washington American Geographical Society of New York.

US EPA. 1985. Ambient water quality criteria for Ammonia. EPA 440/5-85-001. 228 pp

US EPA. 1994. Water quality standards handbook: second edition, Water quality standards branche. Office of science and Technology. U.S. Environmental Protection Agency.

US EPA. 2000. Water quality standards. 40 CFR Ch. I (7-1-00 Edition).

U.S.EPA. 2002. Implementation Guidance for Ambient Water Quality Criteria for Bacteria. Draft. Washington DC. U.S. Environmental Protection Agency. 104 pp.

USEPA. 2000b. Guidance for assessing chemical contaminant data for use in fish advisory. Volume I: fish sampling and analysis, 3nd edition. U.S. Environmental Protection Agency, Office of Science and Technology, Office of Water, Washington DC: EPA 823-B-00-007