Bull Environ Contam Toxicol (2009) 83:35–41
DOI 10.1007/s00128-009-9726-4

Liver Histopathology in Brown Trout (*Salmo trutta* f. fario) from the Tinhela River, Subjected to Mine Drainage from the Abandoned Jales Mine (Portugal)

J. Carrola · A. Fontaínhas-Fernandes · P. Matos · E. Rocha

Received: 18 May 2007 / Accepted: 8 April 2009 / Published online: 24 April 2009

Abstract The objective of this study was to compare the occurrence of toxicopathic liver lesions in brown trout (*Salmo trutta* fario) from Tinhela River near the Jales Mine, both before implementation (2002) and after completion of the governmental mitigation program (2006). Fish were caught in April 2002 and May 2006, using an electrofishing system at four sites: S0, reference station; S1, S2 and S3 as contaminated stations. In 2002, the hepatosomatic index (HSI) was significantly higher for trout captured at the contaminated sites S2 and S3 than in S0. After the rehabilitation program, the HSI of fish sampled at the contaminated sites did not differ from the reference group. The liver of trout caught at S0 exhibited the normal parenchymal and stromal architecture described for the species and there were no pathological abnormalities. In contrast, fish sampled at S3 and S2 sites had diverse toxicopathic alterations. Specifically, livers from the two contaminated sites showed bile duct hyperplasia, often with mild epithelial dysplasia and fibrotic adventitial sleeve, foci of smaller and more basophilic hepatocytes and foci of hepatocellular necrosis; the latter conditions were frequently associated. Compared with the reference animals, increased hepatocellular vacuolization was found in livers from the polluted sites. Histopathological examination revealed differences among sampling sites in the severity and diversity of hepatic lesions clearly related to the proximity of the tailings. No pathological alterations were observed in the livers of brown trout caught in the same four areas of the Tinhela River after the mitigation program in 2006. In conclusion, our results supported that drainage from the abandoned Jales Mine had deleterious toxicological effects in brown trout. Our data suggested that the governmental mitigation program may have reduced the impact of Jales tailings.

Keywords Liver · Histopathology · *Salmo trutta* · Jales Mine (Portugal)

J. Carrola · A. Fontaínhas-Fernandes (✉) · P. Matos
Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Apartado 1013, 5000-911 Vila Real, Portugal
e-mail: fontain@utad.pt

P. Matos · E. Rocha
Interdisciplinary Centre for Marine and Environmental Research, CIMAR Associate Laboratory, Laboratory of Cellular, Molecular and Analytical Studies (CIIMAR), Rua dos Bragas, 289, 4050-123 Porto, Portugal

E. Rocha
Laboratory of Histology and Embryology, Institute of Biomedical Sciences Abel Salazar (ICBAS), Largo Prof. Abel Salazar, 2, 4099-003 Porto, Portugal

Metal mining activities have been linked to serious impacts on the environment, which often persist for several decades after metal exploitation has ceased (Brumbaugh et al. 2005). Metal pollution resulting from mining operations has well-known negative effects on water quality, as well as the flora and fauna, reducing biodiversity and impairing beneficial uses of surface and groundwater (Amisah and Cowx 2000; Gemici 2004; Brumbaugh et al. 2005; Concas et al. 2006). Mine drainage is a complex issue, because it incorporates different stress factors and mechanisms (acidity, rocks reactivity, chemical reactions of acid generation, microbial catalysis and leaching of the weathering products, and chemical toxic effects from metals), all of which can affect direct or indirectly the aquatic ecosystems. The effects of contamination from inactive and/or abandoned mine sites occur at all levels of biological organization, and there are several potential and specific indicators that can be studied at each level (Clements 2000; Solà and Prats 2006).

Gold mining in the Portuguese Jales area dates back to Roman times, and gold has been extensively exploited from 1933 to 1992. During this period, 25 tons of gold and 100 tons of silver were recovered (Santos-Oliveira and Ávila 1995). On the site, 320,000 m^3 of mine residue (containing high concentrations of As, Cd, Cu, and Zn) were deposited on 14.4 ha of tailing area (Bleeker et al. 2002; Mench et al. 2003). Abandoning mining activities and tailings maintenance significantly increased tailing erosion and pollution of water bodies. The Tinhela was one of the rivers that were subjected to prolonged mineral drainage. As a persistent source of chemicals, contaminated sediments in surface waters pose a risk to human and environment health. Several experiments were made to evaluate the toxicity of soils from the Jales tailings, as well as the remediation and vegetation of this area (Loureiro et al. 2005). However, no studies related the impact of trace elements from the abandoned Jales Mine on local aquatic ecosystems, namely in fish.

Heavy metals occur naturally in the environment in trace amounts and most of them are essential for the normal metabolism of fish, but high concentrations induce direct toxicity (Schmitt et al. 2007). The amount of heavy metals in environmental compartments is significantly increased by several anthropogenic activities, such as mining and mineral processing operations. It is known that metals can be taken up by fish from water, food, sediments and suspended particulate material (Hardersen and Wratten 1998; Bu-Olayan and Thomas 2005). Sub-lethal concentrations of metals cause multiple negative effects in wild fish and impact, practically all vital functions, with consequent alterations being seen, for example, at biochemical or histological levels (Amin et al. 2003; van der Oost et al. 2003; Hansen et al. 2007).

Liver plays an important role in vital functions, basic metabolism and accumulation, transformation and excretion of contaminants (Moon et al. 1985; Triebskorn et al. 1997). Liver histopathology is a biomonitoring tool that provides assessment of environmental stressors effects on wild fish populations, and it was even proposed to be one of the most reliable indicators for fish health impairment caused by anthropogenic activities (Hinton and Laurén 1990; Fernandes et al. 2008).

Portuguese authorities have recently ended a mitigation working program at Jales Mine, for minimizing its adverse impacts on river environment and air quality. The aim of this program was to modify the tailings deposit area, reorganizing it in different layers with lesser slope and covering the tailing with special geotextiles and geomembranes to limit access of both water and oxygen to the tailings. Later on, all area was covered with not contaminated soil to allow plants growth, and reduce erosion and negative visual impacts. Remediation efforts planned and implemented in the Jales area are dictating needs for monitoring, including biomonitoring, for gathering solid data to support and assess the progress in mitigation efforts and to identify any environmental impacts of remediation.

With regard to this mitigation program, the goal of this study was to utilize histopathology to assess the occurrence of toxicopathic liver lesions in endemic brown trout (*Salmo trutta* fario) from Tinhela River, before implementation (2002) and after completion (2006) of the governmental mitigation program. In this vein, this study contributed to assess and describe the potential negative biological effects exerted by the toxic mine drainage and the benefits on fish health status derived from the rehabilitation program.

Materials and Methods

The sampling stations selected for this study were located along the Tinhela River, close to the abandoned Jales mine in the northeast of Portugal (Vila Pouca de Aguiar, N41°47′40″; W07°57′87″). One of the four selected sites was upstream of the mine (reference station, S0) and the other three were downstream of the tailings drainage (S1, S2 and S3) (Fig. 1). S1 was the more heavily contaminated site and was located nearest the mine, with greater chemical stress. Sites S2 and S3 were identified as contaminated sites and were located below the tailings. These areas were chosen because they were the most directly affected by the mine tailings and were free of other pollution sources, such as industrial or urban sewage.

Brown trout specimens were collected during the first week of April 2002, prior to the spring runoff, when this trout is reproductively quiescent. Animals were also collected in May 2006, after the conclusion of mitigation works. Fish were caught from the entire stream reach, according to time and distance criteria, using pulsed DC backpack electrofishing equipment with a DC-500 V generator. Sampling time ranged from 45 to 90 min, depending on stream size and complexity. The objective was to collect a representative sample of fish assemblage by methods designed to sample all except very rare species. For the histopathology analysis, only fish of comparable size (10–20 cm) were selected, randomly, from the electroshocked sample at each site. By gonad analysis, the fish were compatible with being late juveniles/young adults.

Characterization of environmental parameters was made at each site according to Cortes (1992). Once caught, fish were anaesthetized with 3-aminobenzoic acid ethyl ester methanesulfonate (MS-222) and their fork lengths and

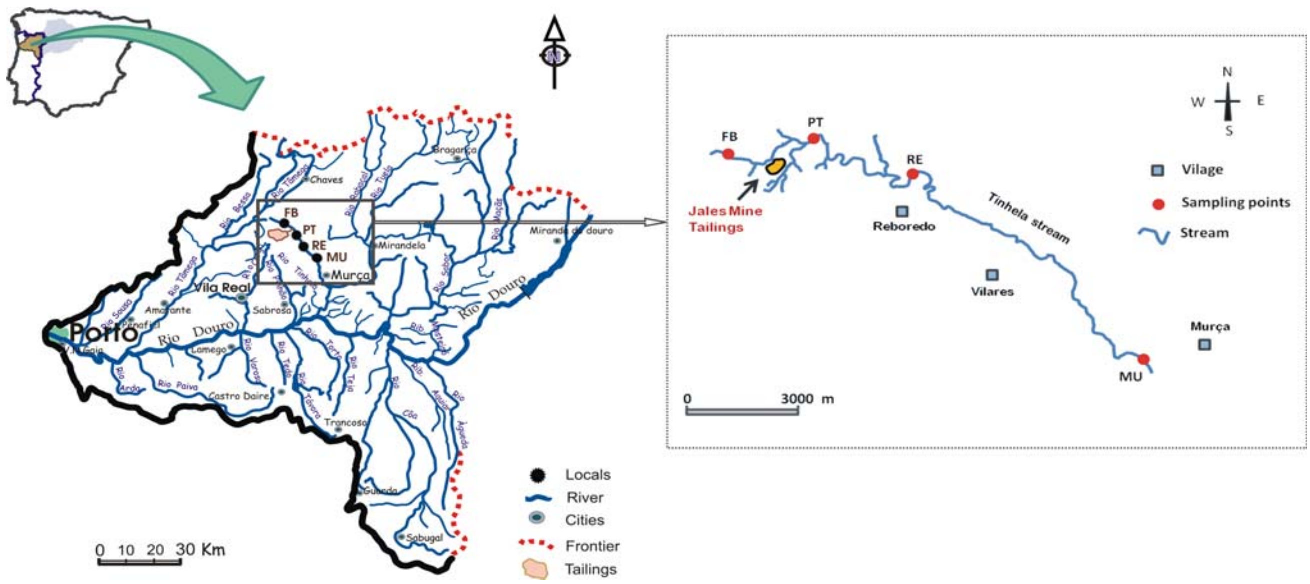

Fig. 1 Map of the study area, located in the Douro Hydrographic Basin, Portugal, showing the sampling sites: Fraga-do-Borralheiro (*FB*, station S0); Peliteira-Tinhela (*PT*, station S1); Reboredo (*RE*, station S2); Murça (*MU*, station S3)

weights were measured. Livers were quickly removed under deep fish anesthesia and weighed before the processing for histological analysis. The hepatosomatic index (HSI) was calculated as: 100 × (liver weight/body weight).

Livers were fixed in Bouin's fixative during 24 h, at room temperature, being cut into 3–4 mm thick slices that were processed for paraffin embedding. Then, 5 μm thick sections were made in a rotary microtome (Leica RM 2135), stained with haematoxylin eosin (H&E), and mounted on glass slides for light microscopy (LM) scrutiny. Sections sampled from diverse blocks were studied. Microphotographies were taken with a Nikon 4500 Coolpix digital camera coupled to a Nikon Eclipse E 600 microscope.

For statistical analysis, it was used the software SPSS 11.0. The HSI and CF data were log transformed to grant normality and homogeneity of variance before ANOVA. Tukey's tests were conducted to compare mean values of HSI and CF between different sites.

Results and Discussion

No trout or other fish were captured at S1 site, the closer local to the mine tailings area. There were no significant differences in body length or weight for fish captured at sites S2, S3 and reference site (S0). Necropsy analysis showed neither external nor internal gross lesions. Furthermore, no skeletal abnormalities were observed on external examination.

Table 1 show that the HSI in 2002 was significantly higher for trout captured at the contaminated sites S2 and S3 than in the reference site S0. In contrast, after the rehabilitation program, the HSI of fish sampled at the former site S2 did not differ from the reference group. As to the CF before the mitigation program, the values from S2 and S3 were significantly different from that in "controls". The CF value reflects the growth and energy reserves of feral fish (Giguère et al. 2004), and the lower CF can be herein associated to the increased metal levels downstream, indicating a chronic exposure and the direct and indirect metabolic impact.

Among other, Mayer et al. (1992) demonstrated that other morphometric parameters, such as the HSI, are sometimes indicative of toxicant effects. Exposure to many kinds of pollutants, like PAHs and PCBs, and also heavy metals, can result in an increase of the HSI of fish, although exposure to others can reduce the HSI when comparing to reference animals. For instance, Norris et al. (2000) observed significant differences in HSI by sex and site, with larger livers (higher HSI) being observed in fish living at the uncontaminated site. Anyway, experimental studies dealing with different pollutants also showed a significantly increase of the HSI in exposed fish (Gadagbui and Goksøyr 1996; Figueiredo-Fernandes et al. 2006a).

Our data may suggest that the higher HSI values in trout at S2 and S3 are connected with exposure to pollutants from mine and tailings drainage, in connection to the metabolic disturbances and/or demand, induced by the toxicants. The causal interpretation for the increased HSI is supported by several authors, despite differences found in other studies. For example, Stephensen et al. (2000) considered that the higher HSI found in sculpin (*Myoxocephalus scorpius*) caught in polluted waters were indicative of

Table 1 Morphological parameters of brown trout caught at the biomonitored sites of the Tinhela River (Portugal) before and after implementation of mitigation program

Sites	S0 (2002)	S2 (2002)	S3 (2002)	S2 (2006)**
Fish (number)	6	8	9	7
Length (cm)	12–18	8–21	9–16	14–22
Weight (g)	17–55	6–63	8–68	26–129
Hepatosomatic index (HSI)*	0.8 ± 0.2^{a}	1.5 ± 0.6^{b}	1.8 ± 0.4^{b}	0.9 ± 0.4^{a}
Condition factor (CF)*	1.3 ± 0.2^{a}	0.7 ± 0.3^{b}	0.8 ± 0.4^{b}	1.5 ± 0.4^{a}

* Values in each row with the same superscripts or no subscripts are not significantly different ($p < 0.05$)

** S2 values from 2006 were compared with control fish caught in 2006. HSI and CF data are presented as mean ± SD

increased hepatic activity and xenobiotic biotransformation with activation of some enzymes. On the other hand, Yang and Baumann (2006) found no trend the HSI of brown bullheads (*Ameiurus nebulosus*) between industrialized and non-industrialized sites in Lake Erie tributaries.

The liver of trout caught at the reference site (S0) exhibited the normal parenchymal and stromal architecture (Fig. 2a) described for the species, including the regular presence of rodlet cells (RC) in the bile ducts epithelia (Rocha et al. 1994a, b, 1995); no pathological abnormalities existed. Hepatocytes showed a homogenous aspect throughout the sections, with a cytoplasm that varied (within normal ranges) from poorly to moderately rich in lipid and/or glycogen deposits, and with a large central or sub central spherical nucleus.

In contrast to S0, fish sampled at S3 and especially at S2 sites consistently had diverse presumptive toxicopathic alterations. Specifically, livers from the contaminated sites showed focal bile duct hyperplasia, often with mild epithelial dysplasia and especially with a thickened (fibrotic) adventitial sleeve (Fig. 2b), also foci of small and basophilic (regenerative) hepatocytes (Fig. 2c), and foci of (often perivascular but also sometimes subcapsular) hepatocellular necrosis (Fig. 2d); with the latter condition and basophilic hepatocytes being frequently associated. In addition, rare inflammatory (essentially lymphocytic) foci and rare distinct (bordered) granulomas also existed (a parasitic nature could not be disclosed), in which scattered extra-epithelial RC coexisted with inflammatory-stromal cellular populations (Fig. 2e, f). Finally, compared with reference fish, a much greater hepatocellular vacuolization was found in the livers from the two polluted sites (Fig. 2g), namely in the S2 station, indicating a trend to mild lipidosis (Hinton and Laurén 1990).

A comment is pertinent about the RC. Whereas the exogenous hypothesis considers RC parasites, the endogenous hypothesis regards them as a genuine fish cell population with a secretory and/or leukocyte function (Mazon et al. 2007). In our study, RC do not seem to have a more regular presence in animals from polluted waters when compared with "control" fish, except in the above cited rare granulomas.

Despite the limited number of trout that could be sampled at the diverse stations, the liver histopathology examination was quite consistent, and clearly revealed differences among sampling sites, such that the severity and diversity of lesions was clearly related with the proximity to the tailings. Hepatocellular necrosis was found only in fish caught at both contaminated sites before mitigation, but it clearly predominated near the Jales Mine, at the S2 site. As referred, no fish were caught in the nearest site from the mine tailings (S1). In our opinion, facing the electrofishing efforts that were made, this occurrence is a consequence of the local deleterious effects of the tailings drainage caused by rain erosion. In fact, there was an obvious decrease in the number of hepatic lesions further downstream from the mine and tailings, strengthening the suspected mine-related effect.

Little is known about liver histopathological changes caused by chronic exposure to mining effluents, despite published data revealing deleterious effects from exposure to selected metals. For example, van Dyk et al. (2007) found in Mozambique tilapia (*Oreochromis mossambicus*) liver lesions that included hepatocyte hyalinization, vacuolation, cellular swelling, and congestion of blood vessels. The intensity of these changes was influenced by the extent of the exposure period to cadmium and zinc.

Moreover, Payne et al. (2001) found several liver lesions in lake trout (*Salmo namaycush*) taken from a large iron-ore contaminated area. Specifically, they found liver perivascularitis, cholangitis, eosinophilic foci and bile duct hyperplasia in lake trout exposed to the metal mining effluents.

The alterations observed in our study are often used as histopathological biomarkers in freshwater fish populations, such as in largemouth bass (*Micropterus salmoides*) caught at contaminated sites (Teh et al. 1997). Liver lesions that solely appeared at polluted locations included bile duct hyperplasia, islands of hyperplastic basophilic hepatocytes, lipidosis, and also the rare granulomas. Besides, the

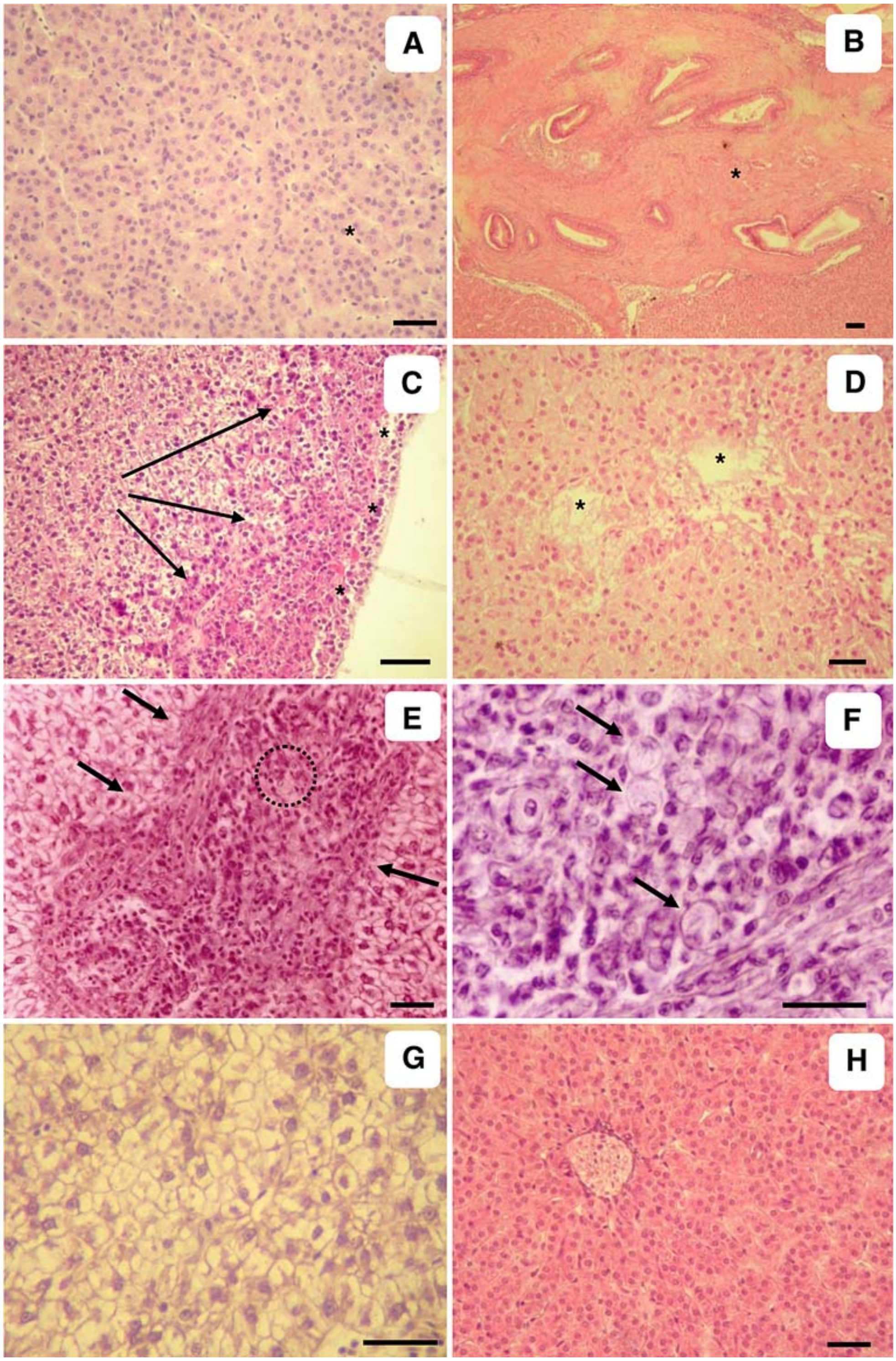

Fig. 2 Liver photomicrographs from brown trout (*Salmo trutta fario*) sampled in Tinhela River before (**a**–**g**) and after (**h**) implementing Jales mitigation program. **a** "S0 liver" illustrating the normal (non-vacuolated) parenchyma; **b** "S2 liver", showing a (non-hilar) focus of mild hyperplasia of bile ducts (BD), with a thick fibrotic sleeve (*); **c** "S2 liver", depicting a band of more basophilic hepatocytes, the outer part of which is associated to some necrosis (*); **d** "S2 liver", with a necrotic focus (*) bordered by distorted parenchyma. **e** Liver with a vacuolated parenchyma and an inflammatory focus with well-defined borders (*arrows*) and a complex cell population within, that includes rodlet cells (*circle*); **f** magnification of the focus in **e**, showing rodlet cells (*arrows*) amidst inflammatory and stromal cells; **g** "S2 liver", showing the parenchyma of fish with general vacuolization; **h** liver sampled after mitigation, with the normal hepatotubular network of the (non-vacuolated) parenchyma being particularly evident, eventually mixed with a multicell thick muralium architecture. H&E, Bar = 10 μm

majority of the histological abnormal changes identified in this study are similar to those observed in fish exposed to chemical contaminants in several field studies (Teh et al. 1997; Stentiford et al. 2003), as well as in our own controlled laboratory experiments with Nile tilapia exposed to diverse pollutants (Figueiredo-Fernandes et al. 2006a, b; Matos et al. 2007). All the above evidence strengthens our interpretation that most of the lesions found herein in brown trout liver had a toxicological aetiology.

No pathological alterations were observed in the livers of brown trout caught in a former polluted area of the Tinhela River after the mitigation program implemented by the local authorities from 2003 to 2006. In fact, all trout caught in 2006 in the S2 station had livers with both a normal stroma and parenchyma (Fig. 2h), thus contrasting with the lesions commonly found in the specimens caught in 2000 in the S2 and S3 stations.

In conclusion, our results strongly supported that drainage from the abandoned Jales Mine had deleterious toxicological effects in local fish, as no fish were caught in S1, and trout captured at S2 and S3 showed an increased HSI, decreased CF and liver changes/lesions, when compared to the reference area (S0). The data reinforces the negative impacts on aquatic ecosystems from exposure to heavy metals. Finally, data from 2006 suggested that the governmental mitigation program may have reduced the impact of Jales tailings erosion. In any case, monitoring and biomonitoring of the Tinhela River using multiple endpoints and indicator species will be continued over the next years to analyze better the impact of the governmental restoration program for the abandoned Jales Mine.

References

Amin O, Comoglio L, Rodriquez E (2003) Toxicity of cadmium, lead and zinc to larval stages of *Lithodes santolla* (Decapoda, Anomura). Bull Environ Contam Toxicol 71:527–534. doi:10.1007/s00128-003-8611-9

Amisah S, Cowx I (2000) Impacts of abandoned mine and industrial discharges on fish abundance and macroinvertebrate diversity of the upper River Don in South Yorkshire, UK. J Freshw Ecol 15:237–249

Bleeker P, Assunção A, Teiga P, Koe T, Verkleij J (2002) Revegetation of the acidic, As contaminated Jales mine spoils tips using a combination of spoil amendments and tolerant grasses. Sci Total Environ 300:1–13. doi:10.1016/S0048-9697(02)00081-5

Brumbaugh W, Schmidt C, May T (2005) Concentrations of cadmium, lead, and zinc in fish from mining-influenced waters of Northeastern Okhlahoma: sampling of blood, carcass, and liver for aquatic biomonitoring. Arch Environ Contam Toxicol 49:76–88. doi:10.1007/s00244-004-0172-3

Bu-Olayan AH, Thomas BV (2005) Toxicity and bioaccumulation of heavy metals *Liza klunzingeri* (Mugilidae: Perciformes). Chem Ecol 21:191–197. doi:10.1080/02757540500117342

Clements W (2000) Integrating effects of contaminants across levels of biological organization: an overview. J Aquat Ecosyst Stress Recov 7:113–116. doi:10.1023/A:1009927612391

Concas A, Ardau C, Cristini A, Zuddas P, Cão G (2006) Mobility of heavy metals from tailings to stream waters in a mining activity contaminated site. Chemosphere 63:244–253. doi:10.1016/j.chemosphere.2005.08.024

Cortes R (1992) Seasonal pattern of benthic communities along the longitudinal axis of river system and the influence of abiotic factors on the spatial structure. Archiv Fur Hydrobiologie 126:185–203

Fernandes C, Fontaínhas-Fernandes A, Rocha E, Salgado MA (2008) Monitoring pollution in Esmoriz-Paramos lagoon, Portugal: liver histological and biochemical effects in *Liza saliens*. Environ Monit Assess 145:315–322. doi:10.1007/s10661-007-0041-4

Figueiredo-Fernandes A, Fontaínhas-Fernandes A, Rocha E, Reis-Henriques MA (2006a) Effects of gender and temperature on hepatic EROD activity, liver and gonadal histology in Nile tilapia *Oreochromis niloticus* exposed to paraquat. Arch Environ Contam Toxicol 51:626–632. doi:10.1007/s00244-005-0208-3

Figueiredo-Fernandes A, Fontaínhas-Fernandes A, Monteiro R, Reis-Henriques MA, Rocha E (2006b) Effects of the fungicide mancozeb on liver structure of Nile tilapia, *Oreochromis niloticus*: assessment and quantification of induced cytological changes using qualitative histopathology and the stereological point-sampled intercept method. Bull Environ Contam Toxicol 2006(76):249–255. doi:10.1007/s00128-006-0914-1

Gadagbui BKM, Goksøyr A (1996) CYP1A and other biomarker responses to effluents from a textile mill in the Volta river (Ghana) using caged tilapia (*Oreochromis niloticus*) and sediment-exposed mudfish (*Clarias anguillaris*). Biomarkers 1:252–261. doi:10.3109/13547509609079365

Gemici U (2004) Impact of acid mine drainage from the abandoned Haliköy mercury mine (Western Turkey) on surface and groundwaters. Bull Environ Contam Toxicol 72:482–489. doi:10.1007/s00128-004-0271-x

Giguère A, Campbell PG, Hare L, McDonald DG, Rasmussen JB (2004) Influence of lake chemistry and fish age on cadmium, copper, and zinc concentrations in various organs of indigenous yellow perch (*Perca flavescens*). Can J Fish Aquat Sci 61:1702–1716. doi:10.1139/f04-100

Hansen B, Rømma S, Garmo Ø, Pedersen S, Olsvik P, Andersen R (2007) Induction and activity of oxidative stress-related proteins during waterborne Cd/Zn-exposure in brown trout (*Salmo trutta*). Chemosphere 67:2241–2249

Hardersen S, Wratten SD (1998) The effects of carbaryl exposure of the penultimate larval instars of *Xathocnemis zealandica* on emergence and fluctuating asymmetry. Ecotoxicology 7:297–304. doi:10.1023/A:1008828600164

Hinton DE, Laurén DJ (1990) Liver structural alterations accompanying chronic toxicity in fish – potential biomarkers of exposure. In: McCarthy JF, Shugart ELR (eds) Biomarkers of environmental contamination. Lewis, Boca Raton, pp 17–57

Loureiro S, Ferreira G, Soares A, Nogueira J (2005) Evaluation of the toxicity of two soils from Jales Mine (Portugal) using aquatic bioassays. Chemosphere 300:168–177

Matos P, Fontaínhas-Fernandes A, Peixoto F, Carrola J, Rocha E (2007) Biochemical and histological hepatic changes of Nile tilapia *Oreochromis niloticus* exposed to carbaryl. Pestic Biochem Physiol 89:73–80. doi:10.1016/j.pestbp.2007.03.002

Mayer L, Versteeg J, McKee J, Folmar C, Graney L, McCume C, Rattner A (1992) Metabolic products as biomarkers. In: Huggett J, Kimerly A, Mehrle M, Bergman L (eds) Biomarkers: biochemical, physiological and histological markers of anthropogenic stress. Lewis Publishers, Chelsea, pp 5–86

Mazon AF, Huising MO, Taverne-Thiele AJ, Bastiaans J, Verburg-van Kemenade BML (2007) The first appearance of Rodlet cells in carp (*Cyprinus carpio* L.) ontogeny and their possible roles during stress and parasite infection. Fish Shellfish Immunol 22:27–37. doi:10.1016/j.fsi.2006.03.012

Mench M, Bussière S, Boisson J, Cataing E, Vangronsveld J, Ruttens A, Koe T, Bleeker P, Assunção A, Manceau A (2003) Progress in remediation and revegetation of the barren Jales gold mine spoil after in situ treatments. Plant Soil 249:187–202. doi:10.1023/A:1022566431272

Moon TW, Walsh PJ, Mommsen TP (1985) Fish hepatocytes: a model metabolic system. Can J Fish Aquat Sci 42:1772–1782

Norris O, Camp M, Maldonado A, Woodling D (2000) Some aspects of hepatic function in feral brown trout, *Salmo trutta*, living in metal contaminated water. Comp Biochem Physiol 127:71–78. doi:10.1016/S1095-6433(00)00236-1

Payne F, French B, Hamoutene D, Yeats P, Rahimtula A, Scruton D, Andrews C (2001) Are metal mining effluent regulations adequate: identification of a novel bleached fish syndrome in association with iron-ore mining effluents in Labrador, Newfoundland. Aquat Toxicol 52:311–317. doi:10.1016/S0166-445X(00)00166-1

Rocha E, Monteiro RAF, Pereira CA (1994a) The liver of the brown trout, *Salmo trutta* fario: a light and electron microscope study. J Anat 185:241–249

Rocha E, Monteiro RAF, Pereira CA (1994b) Presence of rodlet cells in the intrahepatic biliary channels of the brown trout, *Salmo trutta* fario Linnaeus, 1758 (Teleostei, Salmonidae). Can J Zool 72:1683–1687. doi:10.1139/z94-225

Rocha E, Monteiro RAF, Pereira CA (1995) Microanatomical organization of the hepatic stroma of the brown trout, *Salmo trutta* fario (Teleostei, Salmonidae): a qualitative and quantitative approach. J Morphol 223:1–11. doi:10.1002/jmor.1052230103

Santos-Oliveira J, Ávila P (1995) Avaliação do impacto químico ambiental provocado por uma exploração mineira. Um caso de estudo na mina de Jales. Estudos Notas Trabalhos IGM 37:25–50

Schmitt C, Brumbaugh W, Thomas W (2007) Accumulation of metals in fish from lead–zinc mining areas of southeastern Missouri, USA. Ecotoxicol Environ Saf 67:14–30. doi:10.1016/j.ecoenv.2006.11.002

Solà C, Prat N (2006) Monitoring metal and metalloid bioaccumulation in Hydropsyche (Trichoptera, Hydropsychidae) to evaluate metal pollution in a mining river. Whole body versus tissue content. Sci Total Environ 359:221–231. doi:10.1016/j.scitotenv.2005.04.007

Stentiford GD, Longshaw M, Lyons BP, Jones G, Green M, Feist SW (2003) Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Mar Env Res 55:137–159

Stephensen E, Svavarsson J, Sturve J, Ericon G, Adolfson-Erici M, Förlin L (2000) Biochemical indicators of pollution exposure in shorthorn sculpin (*Myoxocephalus scorpius*), caught in four harbours on the south-west coast of Iceland. Aquat Toxicol 48:431–442. doi:10.1016/S0166-445X(99)00062-4

Teh S, Adams S, Hinton D (1997) Histopathologic biomarkers in feral freshwater fish populations exposed to different types of contaminant stress. Aquat Toxicol 37:51–70. doi:10.1016/S0166-445X(96)00808-9

Triebskorn R, Köhler H, Honnen W, Schramm M, Adams S (1997) Induction of heat shock proteins, changes in liver ultrastructure, and alterations of fish behaviour: are these biomarkers related and are they useful to reflect the state of pollution in the field? J Aquat Ecos Stress Recov 6:57–73. doi:10.1023/A:1008224301117

van der Oost R, Beyer J, Vermeulen N (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13:57–149. doi:10.1016/S1382-6689(02)00126-6

van Dyk JC, Pieterse GM, van Vuren JHJ (2007) Histological changes in the liver of *Oreochromis mossambicus* (Cichlidae) after exposure to cadmium and zinc. Ecotoxicol Environ Saf 66:432–440. doi:10.1016/j.ecoenv.2005.10.012

Yang X, Baumann P (2006) Biliary PAH metabolites and the hepatosomatic index of brown bullheads from Lake Erie tributaries. Ecol Indic 6:567–574. doi:10.1016/j.ecolind.2005.08.025