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RISK AROUND THE WORLD

Plan for an Integrated Human and Environmental Risk Assessment in the S. Domingos Mine Area (Portugal)

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ABSTRACT

The need for the integration of the assessment of human and ecological risks in contaminated areas, such as derelict mines, widely increases. The risk assessment process is becoming a powerful tool to provide sound scientific bases for decision-making processes. In Portugal, the risk assessment process is in its early years and the lack of multidisciplinary teams of experts is frequently mentioned as the main obstacle to its implementation. Therefore, the majority of the reclamation actions are based on impact assessment studies that usually are characterized by few biological and toxicological considerations. In order to account for some of these constraints, the ecological risk assessment framework proposed by the U.S. Environmental Protection Agency was used to plan the assessment of human and ecological risks posed by the high concentrations of metals scattered in the vicinity of S. Domingos mine, a cuprous pyrite mine located in the Southeast Alentejo (Portugal). This study presents the problem formulation phase of the assessment. It includes all the scientific information available for the area, a conceptual model, and an analysis plan for the risk assessment process. Following a tiered approach, several tasks were planned in order to acquire chemical, toxicological, and ecological information, in order to compensate for the lack of toxicity data for site-specific species.

Key Words: contaminated sites, heavy metals, conceptual model, analysis plan, human health risk assessment, ecological risk assessment.

INTRODUCTION

Historically, abandoned mines are one of the most serious environmental problems faced by many countries all over the world and Portugal is not an exception.

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The mining industry is probably the anthropogenic activity that produces the deepest impacts on the environment because it is responsible for a complete transfiguration of the landscape and temporary elimination of the vegetation (Starnes and Gasper 1995). It also produces great amounts of solid, liquid, and gaseous waste materials (UN/DTCD and DSE 1992). These impacts usually start in the phase through several physical, chemical, and technological processes performed to treat the raw material. Impacts quite often persist after closure because adequate reclamation measures were not considered. Only in 1990 did European and Portuguese legislation establish that new projects regarding the exploitation and exploration of geologic resources must include a description of the measures adopted to prevent environmental pollution and to ensure restoration after closure. It was also established that explorations involving areas or an annual production greater than 5 ha/150,000 ton, respectively, need to assess potential impacts on human health and on different environmental compartments (soil, water, air, landscape) (Rodrigues 1998).

Particularly within the mining industry, areas where polymetallic sulfides were extracted are important point and non-point sources of heavy metals. The piles of mine tailings (mainly broken and crushed rocks) left in the vicinity, when exposed to air and water, give rise to the oxidation of remaining sulfides, through chemical, electrochemical, and biological reactions, to form ferric hydroxides and sulphuric acid combined in acidic mine drainage (Cohen and Gorman 1991; Merson 1992; Evangelou and Zhang 1995; Larocque and Rasmussen 1998; Soucek *et al.* 2000). Furthermore, during the recharge of the open pit, water percolates to emerge later as a seep or a spring, which is very acidic and will flow even when drought conditions dry up surface waters (Starnes and Gasper 1995). Under acidic conditions, metals may be dissolved in water and transported from the mine site to a receiving stream or water body, or can leach into the groundwater (Cohen and Gorman 1991; Martyěák *et al.* 1994; Sanchez *et al.* 1994).

The soil and sediment fine fraction is also usually enriched in metals, due to the relative large surface area of fine particles for adsorption and due to metal binding to iron and manganese oxides and to organic matter (Rasmussen 1998; Yukselen and Alpaslan 2001). Wind-blown dusts generated in those soils can be responsible for the atmospheric transport of trace metals (Rasmussen 1998). Therefore, soils and sediments are important sinks of heavy metals that could be inhaled, ingested, or absorbed, thereby entering the biosphere (Larocque and Rasmussen 1998).

In short, two kinds of risks, considering their nature, should be analyzed in abandoned mine areas: physical and chemical risks (Aduvire 1998). Physical risks are those posed by the possible collapse of tunnels, galleries, and old buildings, as well as the risks associated with mine pits and lakes, filled with highly acidic waste waters. These risks are greater for children that play on the area or to the adventurous visitor. Chemical risks are produced by the potential exposure of humans and wildlife species to high concentrations of heavy metals present in the different environmental compartments. Once more, children represent a vulnerable population because behaviors such as hand-mouth activity increase likely exposures through ingestion or inhalation (Victorin *et al.* 1999; Casteel *et al.* 2001).

About eighteen derelict mine areas were mapped in the Portuguese territory, as part of a restoration project developed by a partnership between the Direcção Geral do Ambiente (DGA), and the Instituto Geológico e Mineiro (IGM), which mediates concessions for the exploration of geological resources. The aim of this management project (the EIMA project) was to perform studies, in order to acquire the needed baseline information, to set priorities in order to ameliorate safety, human health and environmental impacts yielded by not reclaimed mines. The decisions were based on criteria such as: (1) the dimension of social problems recorded in the area; (2) the amount and quality of available technical information, and (3) the risks posed to the health of local populations (Costa and Leite, 2000).

According to the preliminary results of the EIMA project, uranium mines and those associated with massive sulfides (present in the Iberian Pyrite Belt) have proved to be the most concerning ones. Following the precautionary principle (Kriebel *et al.* 2001), some reclamation measures were planned and will be implemented in those areas, without any prior risk assessment being performed. The lack of a multidisciplinary team of experts in risk assessment and the high economic costs involved were the main constraints pointed out by Costa and Leite (2000) on conducting a risk assessment process. In fact, Portugal is only now starting to develop specific procedures and methodologies for the assessment of contaminated areas (Ferguson *et al.* 1998).

Our interest was focused on the latter kind of mines mentioned earlier because it includes the majority of Portuguese contaminated sites. The S. Domingos mine, in Southeast Portugal, was the area chosen for this study, based on the following criteria: (1) the inclusion of the mine in the first ten priority areas, according to the EIMA project; (2) the significant amount of scientific information available related to biological and toxicological characteristics of the area, and (3) the coexistence of some residential areas with the sources of contaminants, which leads to the suspicion of potentially significant exposure of humans to toxic metals.

The main objective of our study was to plan an integrated assessment of human health and ecological risks associated with surface waters, sediments, and soil in the S. Domingos mine area using already developed frameworks (USEPA 1998a; NAS 1983 *in* Roberts and Abernathy 1996). This article presents the information available for the area and evaluates its suitability to support the objectives of the risk assessment. It also defines the assessment endpoints, the conceptual framework for the assessment, and gives a description of the analysis plan, following a tiered approach, designed to satisfy some critical information needs.

STUDY AREA

Mina de S. Domingos (MSD) (Mértola, Portugal) is a small village, with the same name of the mine, located in Southeast Portugal (about 240 Km from Lisbon), on the left margin of Guadiana River bank, near the Spanish frontier (14 Km) (Figure 1). The study area extends from Corte do Pinto (CP), a small village, 5 Km North of the mine, to Santana de Cambas (SC), in the South, located between MSD and Pomarão (POM) (Figure 1). From Santana de Cambas (SC) toward the South, the impacts

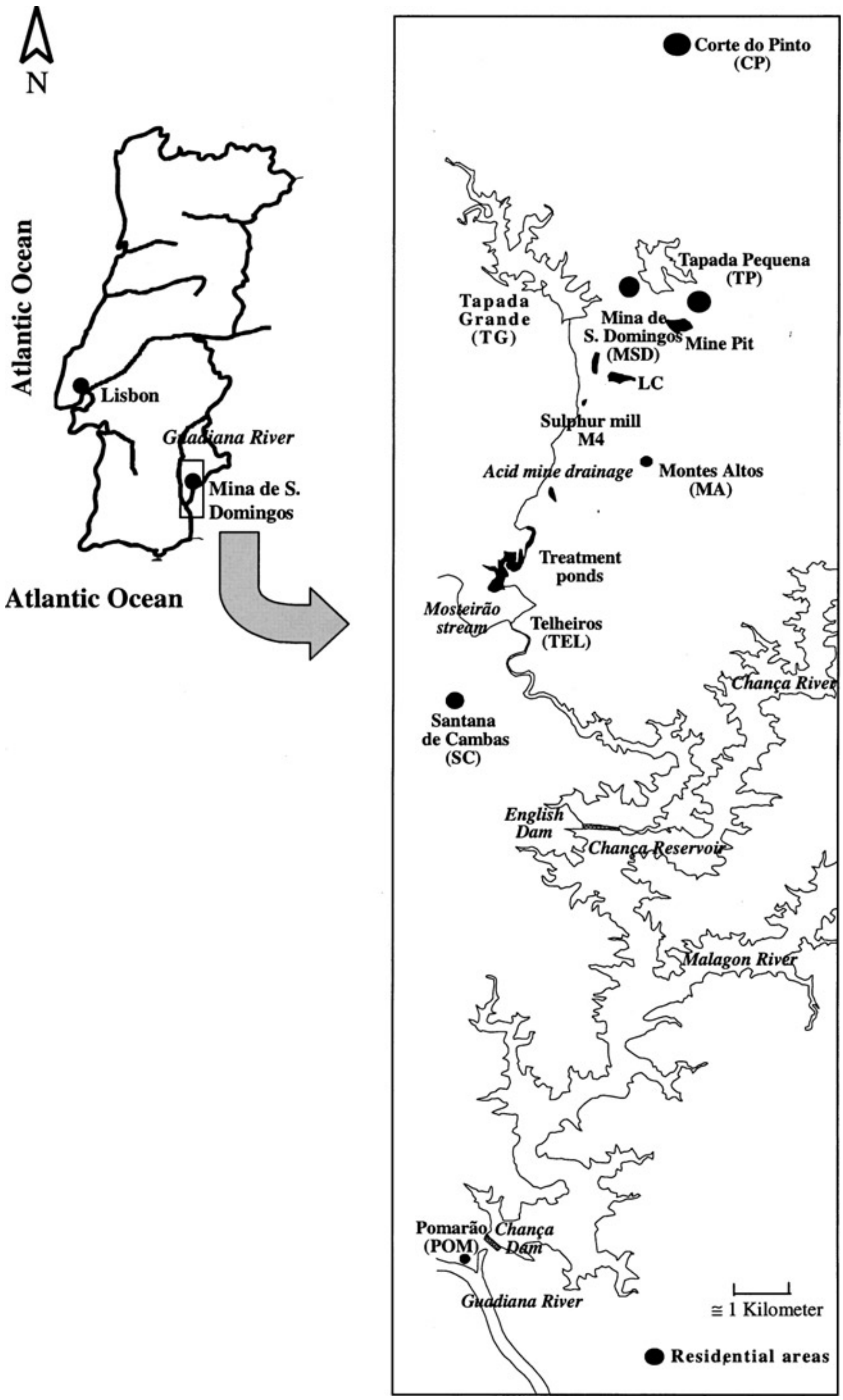

Figure 1. Map of the study area.

were limited only to those produced by ore transport, in a belt of about 20 m wide, following the railway.

Tapada Grande (TG), Tapada Pequena (TP), and Chança River reservoir were also included in the analysis (Figure 1). The former two are freshwater reservoirs built by the mine company to guarantee the supply of high-quality water to mining activities (Pereira *et al.* 1993). Nowadays, Tapada Grande (TG) is highly demanded, mainly by local and regional inhabitants for recreational activities such as canoeing and swimming. Some facilities and substructures were built on its banks to offer satisfying recreational experiences.

Chança River joins Guadiana River, near the Pomarão (POM) village, in a point where the Spanish government built a dam in 1985. The subsequent change imposed in the flow regime has resulted in the formation of an upstream reservoir. The Chança Reservoir submerged the English Dam, a dam built by the mine owners in order to separate the mine hydric system from Chança River (Figure 1). The river and the reservoirs also provide drinking and irrigation water, fisheries, wildlife habitat, and aesthetic pleasure.

According to the main biogeographic divisions, the Portuguese continental territory is located on the Holoartic kingdom and the South Alentejo region, in particular, is within the Mediterranean region whose macroclimate is remarkable influenced by two great continental plates, the Eurasian and the African (Rivas-Martínez 1987). Thus it is characterized by dry, hot summers and mild and rainy winters. Mean temperatures in the summer range between 21 and 25°C. Maximum temperatures frequently exceed 40°C during July and August. Total annual precipitation is low, ranging between 400 and 600 mm and average solar radiation usually attains 3,000 hours of sun per year (CCRA 1994). Particularly in the left margin of the Guadiana River the climate is semiarid, characterized by high seasonal temperature ranges. A dry period of about three to four months is often recorded, during which water level in small and medium-sized rivers and streams is reduced almost until dryness for three or more months every year (Almaça 1995; Pena and Cabral 1996). *Sclerophyllum* species dominate vegetation communities associated with this kind of climate, because they present several morphological and physiological adaptations to reduced levels of available soil water and high evapo-transpiration rates (Pena and Cabral 1996). The area surrounding S. Domingos mine is mainly covered by *Eucalyptus camaldulensis* Dehnh, whose plantation was ordered by the mine owners and engineers in an attempt to reduce atmospheric pollution problems and to stabilize Tapada Grande (TG) and Tapada Pequena (TP) banks. Shrubby vegetation patches, named "garrigues," also exist and are dominated by *Cystus ladanifer* L., *C. psilosepalus* Sweet, *C. salvifolius* L., *C. crispus* L., and *Halimium viscosum* (Willk) P. Silva. Some specimens of *Quercus rotundifolia* Lam., sparsely scattered, and grassland areas are also present in the area. Toward Corte do Pinto (CP) and in the Chança Reservoir banks, the vegetation is dominated by *Quercus ilex* L. and *Quercus suber* L. plantations. With respect to the fauna, the S. Domingos mine area is characterized by a high vertebrate species diversity. Between 1993 and 1995, 128 species of vertebrates excluding bats were recorded by Ribeiro *et al.* (1995), of which 25 and 27 are protected by Portuguese (SEADC and SNPRCN 1990a, b; MARN 1991) and European Union legislation (EC 1979, 1992), respectively. The area is also known for its big game and upland bird hunting. Hunting reserves occupy 60% of the municipality's territory and these resources are responsible for a great influx of people to the area (Pereira 1995).

SITE HISTORY

The ore body of the S. Domingos mine was composed mainly by massive cupriferous pyrite from the Iberian Pyrite Belt. This metallogenetic province is one of the major mineralizations of polymetallic sulphide in the world and one of the major European deposits of basic metals, extending for about 200 Km across Portugal and Spain (Webb 1958; Costa and Goínhas 1988; MPAT and CCRA 1988; Nunes *et al.* 1994; Carvalho 1999). It was formed during the upper Paleozoic and its main deposits, in the form of sheets or bands, are intruded within sequences of volcanic (porphyries and diabases) and sedimentary rocks (Webb 1958; Carvalho 1999). The massive ore body of the S. Domingos mine, with 45 to 48% of sulphur, is primarily in the form of pyrite (FeS_2). Chalcopyrite ($CuFeS_2$), sphalerite (ZnS), galene (PbS), and blend [(Zn, Fe)S] were other minerals recorded in association with pyrite (Oliveira and Oliveira 1996). Within the ore body, the proportion of metals such as copper, lead, and zinc varied widely, ranging up to 7% for copper and 14% for lead and zinc combined (Webb 1958). Strataform manganese deposits, mainly formed by pirolusite (MnO_2), are also frequent in the Iberian Pyrite Belt (Oliveira and Oliveira 1996).

In 1859, a British society, named Mason and Barry Ltd., restarted the contemporary activity of the S. Domingos mine (Alves 1996; Guimarães 1996; Custódio 1996). In a region characterized by a nutrient-depleted soil, cattle breeding was the main socioeconomic activity. Almost one century (1859–1957) of prosperous industrial activity elapsed, during which about 20,000,000 tons of cuprous pyrite were extracted and exported to England (Webb 1958; CMM 1994a; Custódio 1996). Meanwhile, concerned with a possible economic recession and with a world war taking place, the mine owners decided to treat the poorest ore first through grinding and ustulation and then through leaching and cementation methods. In addition, seven dams were constructed to provide settling basins for wet procedures. The high sulphur content (45 to 48%) of the ore also lead to the exploration of this product to supply the national sulphuric acid industry (Custódio 1996; Guimarães 1996). The production and deposition of sulphured smokes and dusts resulted in crop losses. Additionally, acidic waters severely damaged fish nets and killed fish, after being spilled into the Guadiana River (Guimarães 1996). As a result, an effluent treatment method through dam up and evaporation was developed in a complex system of channels, dams, reservoirs, and sedimentation basins or lakes built by the company (Pereira *et al.* 1993).

In 1965, the S. Domingos mine complex shut down its operation after the mining activity became unproductive (CMM 1994a; Custódio 1996). Old mine structures in a high level of corrosion, ruins of industrial buildings, a deep pit filled with highly acidic water, dams, and numerous diverting and channelling streams with characteristic reddish-yellow banks and tons of mining tails are the inheritance left for the old miners and their descendants. The village Mina de S. Domingos (MSD) still persists, with about 800 inhabitants (INE 1991; CMM 1994b; Alves 1996).

PROBLEM FORMULATION

Nowadays, Mina de S. Domingos, similar to almost all of the South Alentejo region, is in a deep economic and social crisis, characterized by high unemployment rates. In order to counteract this situation, local authorities proposed a Urbanization General Plan (UGP) for Mina de S. Domingos and Pomarão (CMM 1994b). Taking advantage of the geographic proximity of Spain and Algarve, the good climatic conditions—especially recorded in spring and autumn—and all the natural and cultural resources available, management goals were established by this plan. These goals included: (1) the promotion of the socioeconomic and urban development of Mina de S. Domingos and Pomarão, always securing the protection of their natural and cultural features; (2) the promotion of tourism and all of its complementary recreation activities (game and aquatic sports), and (3) the improvement of the old mine area and its industrial archaeology through the restoration of the old buildings and of the railway, in order to develop cultural and educational activities.

In agreement with some of the goals established by the UGP, part of the area of the Mina de S. Domingos was integrated in a protected natural area—the Natural Park of the Guadiana Valley (40,000 ha)—created in 1995 (MARN 1995). The main objectives for the establishment of this particular area were: (1) to protect, through the adoption of adequate management strategies, the valuable geomorphological, wildlife, and flora resources; (2) to preserve the historic patrimony and regional traditions, and (3) to promote the sustainable economic development of the local populations (MARN 1995).

In brief, all the management objectives and strategies defined for the Mina de S. Domingos are directed to promote the multiple uses and benefits offered by local and regional resources in order to stabilize the local population and, simultaneously, to attract an increasingly higher number of tourists. However, it is important to have a right notion of the degree of exposure and the subsequent potential effects, yielded by environmental contaminants, to which the local inhabitants and visitors could be subjected. This information is especially important when dealing with a population that permits the use, by the local authorities, of mine wastes to pave the streets, that considers the mine as the main regional value, and that still has expectations about is reactivation (Pereira unpublished data).

Regarding the main objective of a high degree of protection, stated by the European Union Treaty, two of the specific principles of the European Community environmental policy are the preventive and the precautionary principle (Thieffry 2000; Kriebel *et al.* 2001). According to the preventive principle, the environmental policy should focus more on the prevention of hazardous situations rather than on their remediation. Derived from this principle, the Council Directive 93/67/EEC of 20 July 1993 (EC 1993) established the principles for the assessment of risks to man and to the environment of substances notified in accordance with Council Directive 67/548/EEC of 27 June 1967, last amended by the Council Directive 97/69/EEC of 5 December 1997 (EC 1967, 1993, 1997). On the other hand, leaving room for the retrospective risk assessment process, the precautionary principle, consecrated on the European Union (EU Treaty, art. 174) and international law, states that when suspicions of potential harm effects, to human health or to the environment exist, preventive actions should be taken in order to reduce risks (EC 2000). These actions should be preferentially triggered by a risk assessment process. However, the weakness or vulnerability of the scientific foundation, due to insufficient data or due to a high degree of uncertainty associated with available data, cannot hinder action. After that, scientific assessment of risks should proceed and a monitoring program should be performed in order to assess the adequacy and effectiveness of the adopted measures (EC 2000).

In contaminated sites, when fitness for use and protection of the environment are the main purposes for conducting an environmental risk assessment, the risks to humans, water resources, soil, and ecosystem structure and function should be assessed (Ferguson *et al.* 1998). In Portugal, no legislative acts were developed concerning methodologies for assessing risks at these sites. Groundwater and surface water quality guidelines were established according to the different uses of these resources (MARN 1998) and they included maximum admissible and recommendable values of organic and inorganic pollutants. However, and unlike other European Union countries (*e.g.*, Scott-Fordsmand and Pedersen, 1995), soil quality criteria have not yet been defined.

Sources of Contamination

In the S. Domingos mine, a highly acidic effluent is still produced through oxidation of mine tailings exposed to water supplied by precipitation or percolation from Tapada Grande (TG) and Tapada Pequena (TP) (Figure 1). This effluent flows until it joins a small stream (Mosteirão stream), and then enters in Chança Reservoir, or is spilled directly into the reservoir, where it follows a bypass channel (Pereira *et al.* 1993).

Contamination of surface waters and sediments with heavy metals in Chança Reservoir and in the hydric system of the mine was documented in early investigations conducted in the area (Pereira *et al.* 1993; Canteiro 1994; Pereira *et al.* 2000). The highest metal concentrations, especially Al, Cd, Cu, Mn, Ni, Pb, Co, and Zn were recorded during the summer period, in the effluent and in the reservoir (Pereira *et al.* 2000). However, only the total concentrations of Cd (0.0268 mg/l), Ni (68 mg/l), Pb (4.6 mg/l), and Zn (12.10 mg/l) were well above the criteria established for the minimum surface water quality (Tables 1 and 2) (MARN 1998). Processes of chemical partitioning of metals, between the water column, sediments interstitial water, and mineral components, could explain the high levels of heavy metals recorded in the surface water of the reservoir. However, no data are available concerning the rates of these processes.

The presence of other classes of pollutants, such as organic pollutants, was not assessed in the area. However, other sources of contamination are practically nonexistent, because there is no industrial activity, agriculture is scarce and rudimentary, and the demographic density of the area is among the lowest in Europe.

Downstream, the confluence of the acid mine drainage with the Mosteirão stream, in Telheiros (TEL), and then with the Chança Reservoir, is responsible for a reduction in acidity and subsequent precipitation of metals in the stream bed (Table 1), probably by the formation of oxyhydroxide hydrates and/or hydroxysulfate complexes of Fe^{3+} and adsorption to clay or organic matter (Martyěák *et al.* 1994). However, as was observed by Pereira *et al.* (2000), the reservoir has a moderately soft water

Table 1. Chemical characterization of water-column samples collected in the hydric system of the S. Domingos mine (Lopes *et al.* 1999; Pereira *et al.* 2000).

	Mine pit	[1]LC lagoon	[2]Effluent	[3]CRES1*
Al	144.8	440.0	11.3	0.116
Cd	0.84	0.483	0.03	0.0006
Co	3.66	4.12	90	<0.03
Cr	0.53	0.156	<0.02	<0.015
Cu	61.25	10.25	1.7	<0.015
Fe	702.5	655.0	1.64	0.03
Mn	116.8	89.0	3.07	<0.03
Ni	1.8	1.98	68	0.03
Pb	0.1	<0.1	4.6	0.003
Zn	145.75	257.5	12.10	0.09
pH	2.46	2.48	7.03	7.72
Conductivity	7630	4316.7	323	281

[1]LC lagoon (see Figure 1); [2]Effluent (Telheiros, after the confluence with Mosteirão stream); [3]CRES1—point of confluence of the effluent with the Chança Reservoir. Total metal concentrations in mg/l (values preceded by the "less than" signal are below the detection limit). Conductivity in μS/cm.

and low alkalinity values, which are insufficient to neutralize the mine effluent. In spite of this, levels of heavy metals in the water column progressively decreased, after successive dilutions, although high concentrations were still being recorded in the sediments (Pereira *et al.* 2000). More recently, Alvarenga *et al.* (2002b) also recorded superficial water contamination upstream in the Chança reservoir, yielded by old mining works in the Chança mine and in other Spanish mines (*e.g.*, Vuelta Falsa mine). According to the results obtained by these authors, manganese was the most concerning element because the total concentrations recorded in water

Table 2. Maximum recommendable values (MRV), maximum allowed values (MAV), and minimum quality criteria for total metal concentrations (mg/l) in surface waters used for different purposes (MARN 1998).

	Water for human consumption		Water for crops irrigation		Minimum quality criteria for surface waters
	MRV	MAV	MRV	MAV	
Element					
As	0.01	0.05	0.1	10	0.1
Cd	0.001	0.005	0.01	0.05	0.01
Cr	—	0.05	0.1	20	0.05
Cu	0.02	0.05	0.2	5.0	0.1
Mn	0.05	—	0.2	10	—
Ni	—	—	0.5	2.0	0.05
Pb	—	0.05	5.0	20	0.05
Zn	3.0	0.5	2.0	10.0	0.5

samples (ranging between 0.025–80.9 g/L) were well above the maximum allowed level in surface waters for crop irrigation and for the production of drinking water (Table 2) (MARN 1998). However, it is important to mention that no captation of water for human consumption exists at this point of the reservoir.

A mine pit and several treatment ponds with highly acidic water and with high conductivity are present in the ore exploitation area. Their waters are yellow-brownish in color and contain high concentrations of metals, mainly Al, Ni, Co, Zn, Cu, Fe, and Mn (Table 1) (Lopes *et al.* 1999). Tons of mining, milling, and smelting wastes are widely spread, without any protection, and have been frequently used to pave the streets in the residential areas. The likely spread of the finest particles from these wastes through wind transportation should not be ignored. Contaminated soils may also be responsible for the leaching of contaminants into groundwater, with pH being the main factor influencing the degree of leaching (Vangronsveld and Cunningham 1998; Järup 1999; Yukselen and Alpaslan 2001; Van Straalen 2002). Subsurface soil and rock contamination was assessed by Quental *et al.* (2002) in the project MINEO. The main objective of this project was to develop hyperspectral methods to obtain information about soil and rock chemical and mineralogical composition. The highest total metal concentrations were recorded in Minas 4 (M4—near the sulphur mill) and Telheiro (TEL) (Figure 1). Arsenium (1903 ± 3683, 1727 ± 2793 mg/Kg), Pb (4784 ± 8997, 1001 ± 1694 mg/Kg) and Sb (601 ± 1366, 236 ± 467 mg/Kg) were the most concerning elements in the aforementioned areas, respectively. Copper (530 ± 1214 mg/kg) and Hg (659 ± 2292 mg/Kg) concentrations were also high in Minas 4 (M4). Fe concentrations were high in all the studied area. These elements were also present in high levels in soil samples collected in S. Domingos mine, near the exploitation area. The most contaminated soils also had low pH and low organic carbon content (Quental *et al.* 2002), which aroused suspicion about metal bioavailability in the area.

The majority of the inhabitants of Mina de S. Domingos (MSD) live about 500 to 1,000 meters North from the open cast and underground mining area. Thus, environmental data about soil contamination is extremely important because an indirect human exposure is probably occurring through the ingestion of vegetables and fruits that grow there, or of breeding cattle that graze there. Sheep meat, milk, and cheese are well appreciated by locals (Pereira unpublished data). According to Victorin *et al.* (1999), food is the most important exposure pathway for persistent organic pollutants and metals that accumulate on the environment.

Terrestrial Resources

The bioaccumulation of metals in leaves of native plants, from the S. Domingos mine area, was recorded by Pires (unpublished data). Plants from the locally common genus *Genysta,* collected near the mine, had concentrations of Al, Fe, Mn, and Zn 2–2.7-fold above the values recorded in a reference area. Cd concentrations showed the same trend, but only with an increment of 1.6-fold. Leaves from the genus *Cistus* also showed Cd and Zn concentrations 3.4- and 2.4-fold higher in the most contaminated area. The capacity of *Cistus ladanifer* L. to bioaccumulate manganese and zinc and its ability to growth in soils with high concentrations of Cd, Fe, and Pb were recently recorded in another pyrite mine from the Alentejo region

(Alvarenga *et al.* 2002a). The potential of this Mediterranean spontaneous species in the phytorestoration of metal contaminated soils should be assessed because these processes can contribute to an effective and durable immobilization of metals in these areas, reducing leaching and bioavailability (Vangronsveld and Cunningham 1998).

Some preliminary results obtained by Pereira *et al.* (1999) showed higher concentrations of Cd and Fe in the liver of rats (*Rattus rattus* L.) captured near Minas 4 (M4). In opposition, Zn levels were higher in animals captured 5 Km North, near Corte do Pinto (CP) (Figure 1).

Aquatic Resources

Acid mine drainage can cause severe impacts on aquatic ecosystems, not only due to its low pH (high concentrations of H^+) but also due to the high heavy metals content (Herrmann *et al.* 1993; Kemble *et al.* 1994). These impacts usually result in the loss of sensitive species, biodiversity and ecosystem integrity, through effects on growth, reproduction, and behavior of individuals (Starnes and Gasper 1995). The toxicity of the S. Domingos mine effluent and of Chança Reservoir water samples was assessed by Pereira *et al.* (2000) using *Daphnia magna* Strauss and *Ceriodaphnia dubia* Richard in three different bioassays: (1) water-column; (2) sediment, and (3) *in situ* bioassays. Sediment samples collected during the summer period in two sites immediately after the confluence of the acid mine drainage with the Mosteirão stream, near Telheiros (TEL), were extremely toxic to *C. dubia*. No test organisms survived in any of the tests performed in the laboratory. Significant effects on growth of the animals were also recorded in the water-column bioassays for these two sites, when compared to the controls. For the same sites, sub-lethal toxicity of water column and sediment pore water samples collected in this station had already been recorded by Canteiro (1994) in laboratory tests performed with *C. dubia*.

In downstream sites, where the effluent joins the reservoir near the English dam, sediment samples did not reveal any toxicity for *C. dubia* (Figure 1). In addition, fertility was higher in sediment bioassays, for the different reservoir stations, when compared with water-column bioassays (Pereira *et al.* 2000). In the opinion of these authors, food resources in sediment samples or an *hormesis* phenomenon were likely explanations for the results recorded. Lowell *et al.* (2000) also postulated a potential hormonal or metabolic stimulatory mechanism triggered by effluents.

During the fall period, fertility proved to be an insensitive endpoint because no effect was recorded in laboratory water-column bioassays with *D. magna* for all the tested reservoir sites. On sediment bioassays, only the most contaminated site showed a significant reduction in fertility. However, a significant increase in the age at first and second broad releases was observed in both water-column and sediment bioassays. In almost all of the fall experiments, reported by Pereira *et al.* (2000), the effects recorded were significantly more pronounced in the *in situ* bioassays. This study also demonstrated the seasonal variability in the physical and chemical conditions of the reservoir.

Lopes *et al.* (1999) characterized the toxicity of the water in treatment ponds and in the mine pit, having made an attempt to discriminate the toxicity due to heavy metals and due to low pH. Water column samples from the mine pit and from the nearest treatment pond (LC) were significantly toxic in *C. dubia* bioassays when compared to controls. Microtox® also revealed LC water as the most toxic (Lopes *et al.* 1999). However, as noted by these authors, some caution is needed in the interpretation of these results because the light output of *Photobacterium phosphoreum* Cohn (presently, *Vibrio fischeri* Beijerinck) can be altered by pH adjustments (Kross and Cherryholmes 1993).

Canteiro (1994) studied the benthic macroinvertebrate communities of the hydric system of the S. Domingos mine and the Chança Reservoir. Tapada Grande (TG) and Tapada Pequena (TP) (reference sites) were characterized by the dominance of the mayfly *Caenis horaria* L. (70.7%, n = 412) and of *Hydrocarina* sp. (47.5%, n = 292), respectively. *C. horaria* was also abundant in Tapada Pequena (TP) (34.0%; n = 209), whereas Naedidae oligochaetes were the second most abundant group in Tapada Grande (TG) (25.2%; n = 148). In the other two reference sites chosen by Canteiro (1994), in the Chança River, *Caenis horaria* was, once more, the dominant *taxon*, with a relative abundance of 72.3% (n = 831) in the most upstream site. In this site was observed the highest benthic macroinvertebrate abundance (n = 1150). In the downstream site, *C. horaria* was replaced by *Ephemera glaucops* Pictet and the invertebrate community was dominated by the family Corixidae (81.4%, n = 306). The presence of *C. horaria* upstream in the Chança River, similar to Tapada Grande (TG) and Tapada Pequena (TP), probably could be explained by the semi-lentic characteristics of the river (Canteiro 1994).

Mayflies were completely absent from the stations assembled in the reservoir. There, the two stations near the point of confluence with the acid mine drainage showed an impoverished macroinvertebrate community, composed only by two and three *taxa*, respectively, and a significant reduction in the number of individuals (n = 5 and n = 7, respectively). Thus, a reduction of 88.8% in *taxa* richness, relative to Tapada Pequena was observed in the two most contaminated sites in the reservoir. A similar scenario was also observed for the other stations in the reservoir.

In summary, and similar to other studies performed on contaminated aquatic environments (Sanchez *et al.* 1994; Gray 1998; Soucek *et al.* 2000), a deep reduction on species richness and abundance, as well as absence of mayflies were observed by Canteiro (1994) in the Chança Reservoir. Although these endpoints are likely to reflect community level effects yielded by the exposure to a complex mixture of metals this suspicion was not well supported by the physical and chemical characterization of either water-column or sediments. In fact, regarding the reference sites chosen by Canteiro (1994) in the Chança River, only in one site were total metal concentrations in the sediment recorded. Furthermore, although this reference site presented lower levels of Zn (177.92 mg/kg) and Co (0.44 mg/kg) when compared with almost downstream sites, its total concentrations of Cr (36.47 mg/kg) and Ni (3.56 mg/kg) were well above those recorded in the point of confluence of the effluent with the reservoir (15.83 and 1.52 mg/kg, respectively) (Table 3). With respect to water column samples, metal concentrations also failed to distinguish reference and contaminated sites. Furthermore, as Gower *et al.* (1994) pointed out, the reduction in species richness could have been incremented by the fact that the *taxa* were not identified until the species. Canteiro (1994) also considered the absence of vegetation on the banks of the reservoir as a possible reason for the low number of recorded *taxa*.

Table 3. Chemical characterization of sediment samples collected in Chança Reservoir in September 1992 (Pereira, personal communication; Canteiro 1994).

	Ref.	CRES1*	CRES2**	CRES4**	CRES3**	CRES5**
Cu	61.83	989.95	80.57	40.44	35.29	54.23
Zn	177.92	807.99	333.14	235.89	172.41	233.76
Fe	27.82	52.12	18.57	28.64	29.56	45.79
Cr	36.47	15.83	21.26	9.39	20.08	50.1
Cd	0.24	0.22	0.24	0.22	1.01	0.25
Pb	4.45	64.12	7.09	0.61	7.18	0.57
Co	0.44	2.6	1.18	0.9	1.01	0.74
Ni	3.56	1.52	1.65	1.11	1.42	1.73
AVS	15.79	25.78	58.62	26.1	15.88	16.41
SEM	2.46	16.77	9.49	3.53	6.21	2.73
SEM/AVS	0.16	0.65	0.16	0.14	0.39	0.17

*CRES1—point of confluence of the effluent with the Chança Reservoir.
**CRES2–CRES5—downstream sites.
Total metal concentrations in μg/g except for Fe (mg/g). AVS and SEM concentrations in μmol/g dry sed.

Regarding functional feeding groups, collectors, which are usually associated with the sediment, were absent from the station near the source of contaminants. Shredders-scrapers were also absent in all the Chança Reservoir sites. According to Farag *et al.* (1998), the feeding behavior is the main determinant of metal bioaccumulation by invertebrates. This leads to the suspicion that the concentrations of metals in all sediment samples from the Chança Reservoir may be toxic to benthic macroinvertebrates and may have contributed to the disappearance of the aforementioned functional groups. The absence of burrowing organisms also seems to confirm sediment contamination. However, it is important to keep in mind that the lack of riparian vegetation in the Chança Reservoir banks, reported by Canteiro (1994), could also have compromised the establishment of shredder and collector populations (Cummins, 1992).

Canteiro (1994) also reported the sub-lethal toxicity to *C. dubia* of sediment interstitial water, collected in the point of confluence of the effluent with the reservoir in parallel with the study of the benthic macroinvertebrates community of the Chança Reservoir. In fact, sediment samples from the reservoir, near the point of confluence with the effluent and in some sites downstream, exceeded the sediment probable effect levels (PELS) for zinc and copper, proposed by Ingersoll *et al.* (1996) and by Smith *et al.* (1996). These values correspond to the heavy metal concentrations above which effects were observed in *Hyalella azteca* 28-d assays. However, in an apparent contradiction with the biological effects observed, all the sites had sediment simultaneously extracted metal/acid-volatile sulfide (SEM/AVS) ratios (molar values) less than 1 (Pereira *et al.* personal communication; Canteiro 1994). Heavy metals can be trapped in the sediments through binding with carbonates, Mn-oxides, Fe-oxides, organic matter, sulfides, and residuals (Yu *et al.* 2001a). AVS mainly comprises free sulfides, amorphous iron monosulfides (FeS), crystalline macknawite, pyrrhotite

(Fe), and greigite (Fe_3S_4) as well as sulfides of other divalent cations, which are released during cold hydrochloridic acid extraction (Di Toro *et al.* 1992; Van den Berg *et al.* 1998; Yu *et al.* 2001b). Sulfides have proved to have the main role in the efficient fixation of heavy metals in anoxic sediments, whereas Mn- and Fe-oxides may regulate the bioavailability of metals in highly aerobic environments (Van den Berg *et al.* 1998). SEM are the amount of heavy metals obtained during AVS extraction. According to the AVS approach, when the ratio SEM/AVS of molar concentrations is smaller than 1, very low concentrations of heavy metals should be available in the pore water, and, subsequently, sediments should not reveal lethal or sub-lethal toxicity (Sibley *et al.* 1996; Van den Berg *et al.* 1998; Simpson 2001). However, this was not observed in the Chança Reservoir and the results were coincident with those reported by O'Connor *et al.* (1998). These authors attributed toxicity recorded in their study to sediment characteristics other than sulphide insoluble metals. Another possible explanation could be the use of an inappropriate sampling device (van Veen grab) in the Chança Reservoir study (Canteiro 1994). This grab sampler did not prevent the mixture of sediments from different depths, leading to the destruction of the AVS vertical gradient and to a subsequent erroneous determination of the SEM/AVS ratios. Subsurface anoxic sediments, usually richer in AVS, could have contributed to the net amount of AVS in the mixed sediment sample and, subsequently, to SEM/AVS ratio less than one. This aspect was observed by Van den Berg *et al.* (1998) in sediment samples collected in the Meuse River, and reinforced by Boothman *et al.* (2001). The latter authors drew attention to the consideration of fine-scale vertical gradients and seasonal variations when using AVS and SEM measurements to assess the bioavailability of metals in natural conditions.

Surface sediments (from the uppermost 6 cm) where benthic organisms usually dwell, could had lower concentrations of AVS. Otherwise, as it was observed by Peterson *et al.* (1996), bioturbation yielded either by benthic invertebrates or by fish looking for food, could also have been responsible for the oxidation of sediments with the subsequent decline in AVS and the release of metals to the interstitial water. However, Wall *et al.* (1996) observed that bioturbation by carps in a closed system resulted in a twofold and eightfold increase in Cd water concentration. Still cadmium did not become available to be bioaccumulated, due to the simultaneous resuspension of sediment particles with high binding capacity. In addition to the variability associated with depth, results of some studies also indicated that AVS and metal bioavailability may change over time. Subsequently, the seasonal cycles of AVS formation and oxidation may determine whether sediments act as sites for the immobilization of metals or as sources of metals to interstitial water, overlying water, and biota (Clements and Kiffney 1994; Besser *et al.* 1996; Van den Berg *et al.* 1998; Boothman *et al.* 2001). In fact, confirming this variability in sediment conditions, and in opposition to the observations of Canteiro (1994) for the sediment interstitial water, toxicity to *C. dubia* was not observed later by Pereira *et al.* (2000) in sediment samples collected in the point of confluence of the effluent with the Chança Reservoir, and in some sites downstream, as it was described earlier.

Fish communities of Tapada Grande, Tapada Pequena, Chança Reservoir, and Guadiana River consisted of several Cyprinidae species, of which the barb (*Barbus microcephalus* Almaça), the Iberian barb (*Barbus comiza* Steindachner) and *Chondrostoma willkommii* Steindachner are endemic Portuguese species that only occur in the

Guadina River basin (Almaça 1995; Ribeiro *et al.* 1995). Chança Reservoir and Guadiana River also support game fish species such as the carp (*Cyprinus carpio* L.), the goldfish [*Carassius auratus* (Linnaeus)] and the largemouth bass *Micropterus salmoides* Lacépède. The last species is commonly used in local gastronomy. According to Almaça (1995), the high level of endemicity of the Portuguese native ichthyofauna should justify special concern for the conservation of inland waters.

Amphibian species, such as the Western spadefoot [*Pelobates cultripes* (Cuvier)], the Iberian frog (*Rana iberica* Boulenger), and the Spanish turtle [*Mauremys leprosa* (Schweigger)], which are rigorously protected by the Council Directive 92/43/CEE of the 21 May 1992 (Annex II and IV) (EC 1992), were recorded in the S. Domingos mine area (Ribeiro *et al.* 1995) and are commonly found in the freshwater bodies and in the reservoir for breeding, laying eggs, and larval development. Mammals such as the wild boar (*Sus scrofa* L.), the fox (*Vulpes vulpes* L.), and the otter (*Lutra lutra* L.), also protected by the same Directive, seek these water resources for eating (large crustaceans, amphibians, and fish) and for drinking (Ribeiro *et al.* 1995; Pena and Cabral 1996). Wild boars also use the reservoirs to wash and take care of their coats (Pena and Cabral 1996).

CONCEPTUAL MODEL AND ANALYSIS PLAN

Previous studies performed in the S. Domingos mine area created concern about chronically low-level exposures that may impact aquatic biota and human health. Moreover, the lack of information about the status of terrestrial biota is also highly concerning because the large amounts of ore residues in the area have produced high concentrations of metals in the soil. These elevated metal concentrations may impact biota, altering soil structure and function. Therefore, based on the available information, assessment endpoints and a conceptual model for an integrated assessment of human health and ecological risk assessment were developed. Information about public interests, expectations, main economical and recreational activities, and attitudes toward management decisions consecrated on the UGP were also collected through a questionnaire delivered in the S. Domingos mine area (Pereira unpublished data). Inputs from the public are very important and helpful to choose socially valuable assessment endpoints (Barton and Sergeant 1998).

The goals of the risk assessment for S. Domingos mine area were:

- to evaluate metal bioavailability on the soil and sediment compartment;
- to assess the likelihood of adverse effects of historical contamination on terrestrial and aquatic individuals, populations, or communities;
- to evaluate human exposure and to determine the most important pathways; and
- to formulate and prioritize clean-up strategies.

Physical risks will not be considered in the plan of this first approach to the S. Domingos mine risk assessment because we believe that the unsuitable and risky use of the area can be controlled immediately by restricting visitors' access to certain areas and buildings, using fences and appropriate advertisements. Additionally, education/information programs can also contribute to shifts in visitors' attitudes.

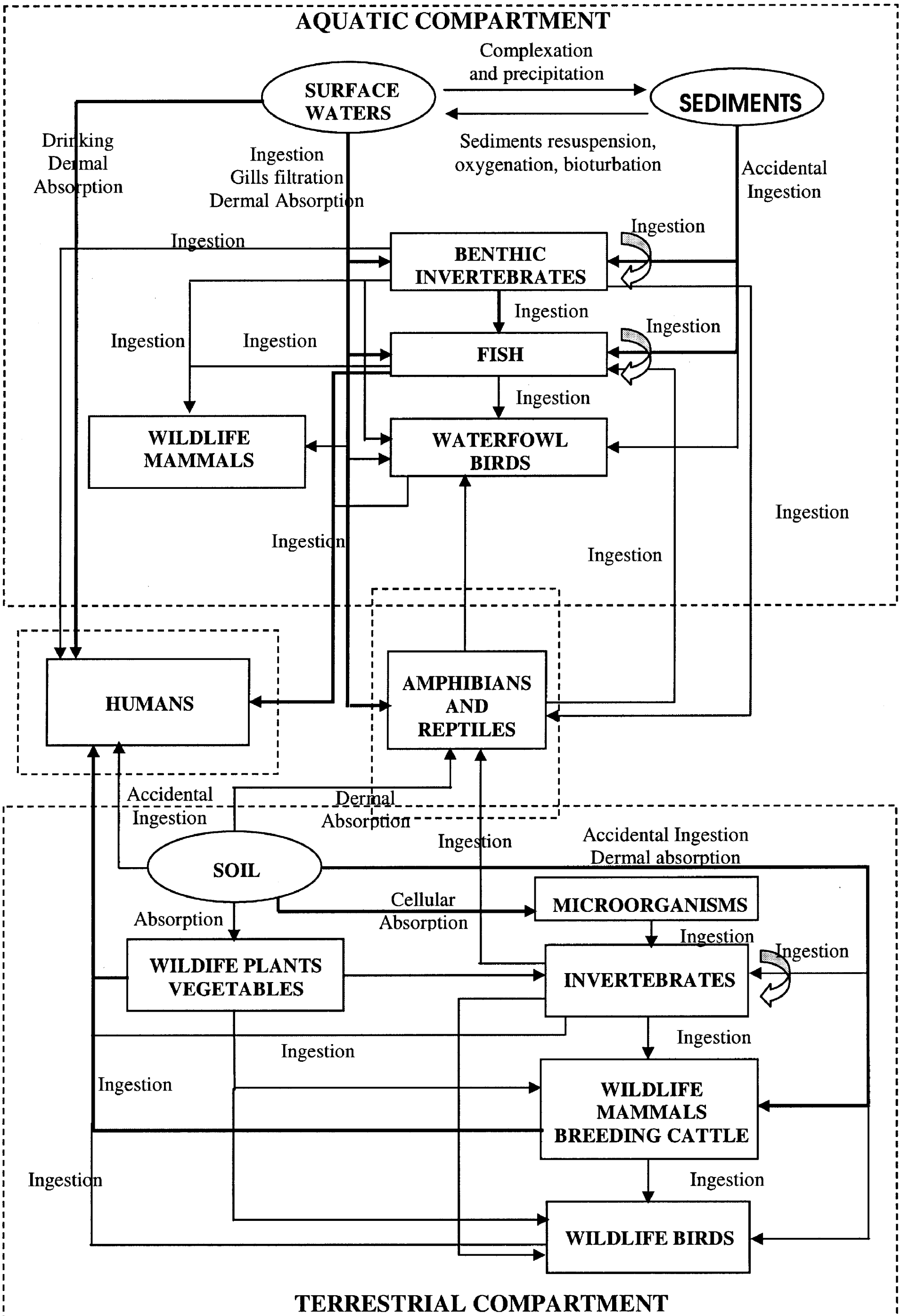

Figure 2. Conceptual model for the S. Domingos mine risk assessment process (the tick lines represent the most important exposure pathways).

The conceptual model included the sources of contaminants, the individuals, populations, and communities of receptors at risk and the likely exposure pathways (Figure 2). The analysis plan for the site-specific assessment of the S. Domingos mine comprised several tasks defined in a tiered approach, as proposed by USEPA (1998b) (Figures 3 and 4). The plan included two tiers. The first one (Tier 1) mainly focused on soil contamination and on the assessment of the terrestrial individuals and communities (tasks 1A to 1F) (Figure 3). Tier 2 included surface water (task 2B) and sediment physical and chemical characterization (task 2C) in conjunction with a seasonal sampling of benthic invertebrates (task 2D), a spatial characterization of fish community structure (task 2F), and an evaluation of fish and amphibian exposure through the analysis of tissues and whole body residues (tasks 2G and H) (Figure 4). Human exposure evaluation was also planned in Tier 1 (task 1F) (Figure 3). The performance of some tasks from tier 2 (*e.g.*, task 2A) depended on the results obtained in previous tasks (Figure 4). Tier 1 focuses on the soil compartment because of the lack of information for the area.

Terrestrial Compartment

The soil is responsible for the transformation, decomposition, and mineralization of organic matter and it also supports a great diversity of living forms. Soil biomass is composed, in a large percentage, by microorganisms, which include bacteria, protozoa, fungi, and algae (Nielsen and Winding 2002). These organisms play an important role in litter decomposition, toxic substances biodegradation, nutrient mineralization from organic matter, and nutrient release from mineral matrices. Therefore, they play a pivotal role in the natural cycles of carbon, nitrogen, phosphorus, sulphur, and, subsequently, to soil structure and quality for plant growth and soil fauna. Additionally, microorganisms have an important contribution in the maintenance of water holding capacity and soil structure through the production of polysaccharides and other cellular debris, that function as cementing agents stabilizing soil aggregates (Gerrard 2000; Nielsen and Winding 2002). Microorganisms are important constituents of all terrestrial food chains, they guarantee a provision of food for soil invertebrates, and they have the ability to provide an integrated measure of soil health (Fairbrother *et al.* 1999; Efroymson and Suter II 1999; Nielsen and Winding 2002).

In order to perform degradative processes of the high molecular weight organic molecules, soil microorganisms release several enzymes into the soil (Rossel *et al.* 1996). These enzymes catalyze several chemical reactions and have a relevant role in the soil microbial processes. Several studies have demonstrated a reduction in soil enzymatic activity due to exposure to heavy metals in contaminated sites (Rowell and Florence 1993; Rossel *et al.* 1996; Kuperman and Carneiro 1997). Furthermore, Rossel *et al.* (1996) have confirmed the suitability of soil enzymatic and soil microbiological investigations, mentioned by Schinner (1996), to assess the intensity and duration of the effects yielded by physical and chemical influences on soil metabolic capacity (Rossel *et al.* 1996). Additionally, enzyme assays have revealed to be sensitive, specific, simple, and cost-effective tools (Rossel and Tarradellas 1991; Rowell and Florence 1993; Sinsabaugh 1994). Therefore, dehydrogenase activity, acid phosphatase activity, and potential nitrification were chosen as measurement endpoints

		Tasks	Measurements
SOIL COMPARTMENT		**Soil physical and chemical characterization**	**A.** - total metal concentrations in soil; - pH, conductivity, organic matter content, moisture content, particle size distribution; cation exchange capacity;
	Soil Function and Terrestrial Biota	**Microorganisms community evaluation**	**B. Microorganisms enzymatic activity** - dehydrogenase activity (Öhlinger 1996b); - acid phosphatase activity (Margesin 1996); - potential nitrification (Kandeler 1996);
		Soil meso and macrofauna community evaluation	**C. Community structure parameters** - abundance/activity; - biological diversity; - number of *taxa*;
		Sub-lethal soil toxicity evaluation	**D.** - seedling emergence, growth, root and shoot length and total wet or dry mass in long-term toxicity bioassays with *Lactuta sativa* and other plant species (OECD 1984; ISO 1993, 1995); - reproduction (number of offspring per female) in *Folsomia candida* and *Eisenia fetida* (ISO 1998; ASTM 1999; ISO 1999);
		Small mammals exposure and effects assessment	**E. Bioindicator species (*Rattus rattus* and *Mus spretus*)** - liver, spleen, and kidney residues; - hair residues; - liver, kidney, thymus and spleen histopathological features;
	Humans	**Local residents exposure evaluation**	**F.** - human hair residues; - questionnaire to collect information about health status, main food items and potential confounding factors;

Figure 3. Tier 1 tasks from the analysis plan of S. Domingos mine area risk assessment.

for the functional characterization of the terrestrial microbial community from the S. Domingos mine area. Nitrification was considered a sensitive parameter because only a small number of nitrifier bacteria are involved (Visser and Parkinson 1992). Due to economic constraints, the assessment of these parameters (task 1B) had be done simultaneously with soil physical and chemical characterization (task 1A) in a single sampling effort (Figure 3). For physical and chemical characterization, especially for heavy metal content evaluation, composite sampling may be considered

		Tasks	Measurements
	Humans	Local residents exposure evaluation	**A.** - food items residues (see task 1F, Figure 3); - drinking water metal contents;
AQUATIC COMPARTMENT		Overlying water physical and chemical characterization	**B.** - heavy metal total and dissolved concentrations, TOC, DOC, pH, conductivity, dissolved oxygen, ammonia, alkalinity and hardness.
		Sediments physical and chemical characterization	**C.** - heavy metals total concentrations; - SEM/AVS ratios, TOC, pH; - sediment particle size distribution;
	Aquatic biota	Benthic invertebrates community evaluation	**D. Community structure parameters** - richness and total abundance; - percent Ephemeroptera abundance; - Ephemeroptera-Plecoptera-Trichoptera richness; - bioaccumulation evaluation;
		Sub-lethal sediments toxicity evaluation	**E.** - survival, growth, female and male emergence, adult mortality, number of eggs produced and number of eggs hatched in sub-lethal toxicity bioassays with *Chironomus riparius* (ASTM 2000; USEPA, 2000).
		Fish community evaluation	**F. Community structure parameters** - relative abundance; - species composition; - standard length, total length, body depth, weight; - incidence of external anomalies, parasitism;
		Fish exposure and effects assessment	**G. Bioindicator species (*M. salmoides* and *Cyprinius carpio*)** - whole body, liver, and gills residues; - histopathological features on liver and gills;
		Amphibians exposure assessment	**H. Bioindicator species (*Rana perezi*)** - whole body residues;

Figure 4. Tier 2 tasks from the analysis plan of S. Domingos mine area risk assessment.

because it has the main advantage of reducing costs and allowing the determination of the mean total concentrations of heavy metals for each area (USEPA 1996; Malherbe 2002; Nielsen and Winding 2002). Methods for soil samples collection (design, depth, and devices), sampling storage, and transportation as well as for soil physical and chemical evaluation are well documented (USEPA 1992, 1996; SPAC 1999).

According to prevailing wind drifts, the reference area chosen for soil communities biosurveys was located at 5 km North from the mining area, in Corte do Pinto (CP) (Figure 1). Another eight sampling sites were selected in the mining area, according to the distance to the exploitation zone, toward the South. Within each sampling site, the different vegetation patches were considered. The choice of a reference area is the most difficult task in these studies. It should have the lowest toxicity of heavy metals as possible, other contaminants should also be absent, and the soil characteristics must be similar to those of the contaminated site (USEPA 1992; Indeherberg *et al.* 1998; Van Straalen 2002). However, as recorded by other authors (*e.g.*, Hudson *et al.* 1997; Alvarenga *et al.* 2002b), high metal contents should be expected in disturbed and undisturbed soils due to natural mineralization. The difference, if one exists, is related to the level of bioavailability of those metals. In summary, as emphasized, the measurement of soil total metal content gives little information because the external available fraction of metals in the soils is determined by soil abiotic factors, such as pH, Eh, organic matter content, clay content and moisture content, hydrous oxides, and manganese oxides, as well as by biotic factors (Ahlers 2001; Peijnenburg *et al.* 1997; Indeherberg *et al.* 1998). However, pH was generally recognized as the most influential factor governing the percentage of mobilizable metals to the solution phase, thus controlling their bioavailability to soil organisms (Gupta 1992; Giller *et al.* 1998; Rieuwerts *et al.* 1998). The methodology for soil sample collection and preservation for enzymatic activity analysis was described by Öhlinger (1996a). It is important to note that permanent changes in soil biological activity are best investigated in spring, before the beginning of the vegetation period. In order to obtain a representative sampling, soil samples (ten replicates) are to be randomly collected in each vegetation patch, within each environmental unit considered (task1B) (Figure 3). Methods for the evaluation of dehydrogenase activity, acid phosphatase activity, and potential nitrification are described by Öhlinger (1996b), Margesin (1996), and Kandeler (1996), respectively.

Because ecological rehabilitation is one of the goals of the reclamation project, soil fauna is very important because it can contribute to a faster recovery at the site and can be used as indicators to evaluate the effectiveness of restoration measures (Haimi 2000). Soil invertebrates, which include four main groups (arthropods, nematodes, annelids, and molluscs), occupy a central role in soil energy pathways, as they feed on all types of organisms and materials and may, subsequently, be exposed to soil and pore water–associated contaminants through dermal uptake and ingestion. These organisms can also be responsible for the transference of bioaccumulated contaminants to the above ground food webs (Fairbrother *et al.* 1999; Gerrard 2000; Haimi 2000). Keep this in mind, the soil invertebrates community of S. Domingos mine area was also considered an appropriate endpoint to assess risks posed by soil contaminants. The soil invertebrates assemblage will be surveyed in four sampling periods (summer, fall, winter, and spring) (task 1C) in the same sampling sites defined for tasks 1A and 1B (Figure 3). Pitfall trapping is the most commonly used method for capture of invertebrates and is a simple and cost-effective method of capturing a very large number of invertebrates with minimum effort (Ausden 2000). According Read *et al.* (1998), canonical correspondence analysis (CCA) proved to be a powerful technique to clarify the relations between soil invertebrate species (using total number of individuals for each species) and environmental variables (*e.g.*, pH, heavy metal total concentrations, soil type, organic matter content) and to make inferences about the effects on soil community structure. Additionally, the evaluation of species diversity may help to understand community structure. Therefore, this procedure will be considered for soil invertebrates and enzymatic activity data collected in the S. Domingos mine area.

Bioassays with at least three different species—a soft-bodied, a hard-bodied invertebrate, and a plant species—should be considered, in order to assess risks due to exposure to soil contaminants from different exposure routes. This is the most appropriate approach when concern with metal bioavailability exists (Yukselen and Alpaslan 2001). Bioassays, which expose organisms to the naturally contaminated soil, collected in the mining area, are the most advisable ones for site-specific assessments because of uncertainties in extrapolating across soil types (Fairbrother *et al.* 1999; Van Gestel *et al.* 2001). *Eisenia fetida* Savigny, *Folsomia candida* Willem, and *Lactuta sativa* L. will be the species considered for sub-lethal and inibition bioassays because standardized protocols have already been established (OECD 1984; ISO 1993, 1995, 1998, 1999; ASTM 1999) (Task 1D) (Figure 3).

Plants are likely taking up contaminants directly from the soil or through the deposition of metal containing dusts on their surfaces, whereas herbivorous wildlife and cattle (mainly sheeplike) consume plant and accidentally ingest soil. Subsequently, herbivorous are exposed to high concentrations of metals because they consume large amounts of vegetation. Therefore, rats (*Rattus rattus* L.) and the Algerian mice (*Mus spretus* Lataste)—an omnivorous and a herbivorous species, respectively, were chosen as indicator species in the S. Domingos mine risk assessment. The use of wildlife animals as monitors or sentinels of environmental hazards has already been reported in several studies (*e.g.*, Talmage and Walton 1991; Hyvärinen and Nygrén 1993; Creekmore *et al.* 1999; Fox 2001; Mertens *et al.* 2001; Nunes *et al.* 2001). These organisms can provide powerful information about characteristics, amounts, and types of chemicals present in the environment, and in many cases they tend to respond more quickly than humans and at lower doses (NRC 1991). Moreover, wildlife species can be potential sources of contaminants to humans or other wildlife species that may consume them (Gilman 1991). Consequently, heavy-metal residues in prey tissues can be used to estimate risks to predators (USEPA 1998a). The selection of rats and of Algerian mice for the S. Domingos mine risk assessment process was based on the following reasons: (1) these species have a large geographic distribution and can be found in contaminated and non-contaminated areas at high population densities (most of the time habiting with man and eating their food); (2) they are a key component of some terrestrial ecosystems and occupy an intermediate middle position in many food chains; (3) they contact soil during their entire life cycle, being exposed to heavy metals by ingestion of contaminated food or soil, or through dermal absorption; and (4) they have small home ranges, typically less than 90 m, which makes them appropriate site-specific indicators of contamination levels at the population levels (MacDonald and Barret 1993). Additionally, wild populations are usually large enough to support harvesting without major adverse effects (NRC 1991).

For the animals that will be collected in the S. Domingos mine area, a histopathological analysis of liver, kidney, and spleen will be performed in conjunction with tissue and hair residues analysis, in order to acquire information about metal bioavailability in the terrestrial compartment and to make some inferences about human and top predator exposures (task1E) (Figure 3). Heavy metals accumulate mainly in the liver and kidney of mammal species, which is explained by the primary role of these organs in metal excretion (Jeong *et al.* 2000; Liu *et al.* 2000; Silva *et al.* 2000). Lipoperoxide formation and cell damage caused by superoxide radicals (O_2^-) may be the main mechanism responsible for tissue lesions in exposed animals (Kotsanis and Llipoulou-Georgudaki 1999). These lesions tend to precede other morphological and biochemical alterations. According to Kålås *et al.* (2000), effects may be expected on wild species at contamination levels well below those responsible for acute effects.

The capture of wild mammals was planned using livetraps that will be be randomly distributed at two sampling sites: North of Tapada Grande (TG)—reference site—and a potentially contaminated site, located in the ore exploitation area. Following the community legislation in force (EC 1986), regarding the protection of animals used for experimental and other scientific purposes, the number of animals captured must be reduced to a minimum.

Aquatic Compartment

In the aquatic compartment, detritus and sediments are consumed by benthic invertebrates and detritivorous fishes. Benthic invertebrates are also potentially exposed to heavy metals, available in sediment pore water, through dermal uptake or gill absorption. These exposure pathways can also be important for fish. Contaminants can either pass through the gills without being taken up, can be excreted through the urine, or transformed and accumulated in different organs (Newman 2000).

It was recognized in planning the risk assessment that the earlier study, on benthic macroinvertebrates communities, performed in the S. Domingos mine area, more precisely in the Chança Reservoir and Tapada Grande (TG), was limited by not accounting for sediment physical differences and for the possible effects of seasonal and depth variations in metal bioavailability. This seriously compromises the validity of the study because these organisms are extremely influenced by annual changes on the bottom substrate and overlying water with respect to parameters, such as dissolved oxygen content, organic matter inputs, and temperature (Cummins 1992; Wetzel 1993). Another gap that could be pointed out to the study of Canteiro (1994) is related to the fact that the validity of Tapada Grande and Tapada Pequena as reference sites was not confirmed by a representative water-column sampling and by the physical and chemical analysis of sediment samples (previously discussed). Still, the benthic macroinvertebrate community was considered an appropriate endpoint for the risk assessment in the S. Domingos mine area because as it was mentioned earlier these organisms are potentially exposed to sediment-associated metals, via all important routes, and they have been shown to bioaccumulate metals (Cain *et al.* 1992; Clements *et al.* 1994; Milan and Farris 1998; Warren *et al.* 1998; Gundacker 2000; Croteau *et al.* 2001). Additionally, benthic macroinvertebrates are perfect integrators between microorganisms and fish, are universally abundant in running waters, easily collected, their life cycles are compatible with seasonal or annual sampling regimes, and they are large enough to be observed without sophisticated instruments (Cummins 1992).

Benthic macroinvertebrates surveys will be performed seasonally in four sampling phases (task 2D), in conjunction with sediment physical and chemical characterization (task 2C) (Figure 4). The latter task will include the assessment of total metal concentrations, TOC and AVS in sediment cores, in order to account for depth variability in these parameters. The assessment will be performed in at least two layers (0–3 cm and 3–6 cm) (Besser *et al.* 1996). For invertebrate surveys, EPA 841-B-98-007 (USEPA 1998b) provides a description of the most appropriate sampling location, the most advisable sampling gear, and also information about sample replication and sample processing. Soucek *et al.* (2000) recorded ephemroptera-plecoptera-trichoptera (EPT) richness and relative ephemeroptera abundance as the two most sensitive endpoints. The most abundant and widespread species in all sampling areas, which are probably the most tolerant species, may be considered to assess its value as a bioindicator of contaminant bioaccumulation (task 2D) (Figure 4).

In order to have a reliable picture of sediment toxicity, sub-lethal toxicity bioassays with *Chironomus riparius* Meigen will be conducted in parallel with the evaluation of natural benthic assemblages (task 2E) (Figure 4). This species, and another from the same genus, have been widely used and have been considered sensitive to several chemicals in whole sediment toxicity assays (*e.g.*, Kemble *et al.* 1994; Sibley *et al.* 1996; Finlayson *et al.* 2000). Guidelines for conducting the aforementioned bioassays are available in the documents edited by USEPA (2000) and ASTM (1990, 2000). The endpoints commonly assessed are survival, growth, female and male emergence, adult mortality, number of eggs produced, and number of eggs hatched (USEPA 2000). Methods for sediment sample collection, handling, storage and preparation, and test procedure are described by ASTM (1990), SETAC (1993), and USEPA (2000) and will be followed in order to minimize sediment disturbance and subsequent changes in bioavailability (task 2E) (Figure 4).

Once in the biota, the contaminants can be passed through the food web, bioaccumulating in carnivorous and omnivorous fish species such as the largemouth bass (*Micropterus salmoides* Lacépède) and the sunfish (*Lepomis gibbosus* L.). This poses a threat to waterfowl species such as the capped heron (*Ardea cinerea* L.), the egret (*Egretta garzetta* L.), the black-crowned night-heron [(*Nycticorax nycticorax* (L.)], and the osprey (*Pandion haliaetus* L.). However, only the capped heron breeds in the area (Rufino 1989).

A fish community structure study (task 2F) was planned for the Chança Reservoir. Sampling will be conducted along transects in at least four stations, one in the mixing zone, two downstream, toward the Chança dam, and a reference station upstream in the river (Figure 4). The stations will coincide with those defined for tasks 2B, 2C, 2D (Figure 4). In each sampling station, a description of the substrate, vegetation patches, water depth, and current will be provided. Fish surveys will be performed using a combination of electrofishing and trawls, in late summer or early fall (USEPA 1993, 1998b). Animals will be identified, measured, and examined for external anomalies in the field, and immediately released in order to prevent harm to sport, endangered, and larger specimens. Additionally, largemouth bass—a predator species—and the carp—a bottom feeder species—were chosen as species to assess metal bioaccumulation on fish tissue, and potential effects through histopathological analysis of liver and gills (task 2G) (Figure 4). Gills are especially important for assessing the effects of surface water contaminants because these organs are constantly in direct contact with this environment. For contaminant analysis and for histopathological examination, replicated composite samples of three to ten fishes will be considered, for each species (USEPA 1993). In order to meet both the human health and the ecological requirements, the largemouth bass and the carp will be filleted and the data used for human health risk assessment. A whole body analysis will be performed for the bottom-feeder species and the data used for ecological risk assessment (USEPA 1993). Methods for sampling, preparation, and storage of fish tissues for contaminant analysis are provided in the EPA 600/R-92/111 document (USEPA 1993).

The potential risks to the amphibian populations are probably very high in the S. Domingos mine area, because they have gilled larvae and in their life stages they possess thin moist skins that may absorb high concentrations of chemicals from the aquatic and terrestrial compartment. After absorption, contaminants will bioaccumulate on different tissues (Harfenist *et al.* 1989) and amphibians can act as a contaminant pathway for fish and bird species that feed on larvae and adults, respectively. The ingestion of frogs by men is not significant in this region of the country.

The effects of pH and toxicants on amphibians were well described by Harfenist *et al.* (1989) and recently updated by Pauli *et al.* (2000). However, few data exist about population-level effects (Gibbons *et al.* 2000). An amphibian community structure study would be relevant for the S. Domingos mine area but it would be very difficult and time consuming, due to the lack of previous data for the region about the populations' temporal variability. These data are very important because this group can be strongly influenced by several environmental conditions (*e.g.*, water regimes, eutrophication) (USEPA 1995, 2003) that may lead to misinterpretations of cause-effect relationships. Therefore, *Rana perezi* Seoane, a widely distributed and abundant species, was chosen as a bioindicator species in order to assess whole body metal concentrations and confirm exposure of amphibians to metals in the S. Domingos mine area (task 2H). The most appropriate methods, devices, and periods to sample amphibians are described in USEPA (1995) and Halliday (2000). Amphibians will be sampled in the Chança Reservoir, Tapada Grande, and Tapada Pequena. In the reservoir, the sampling sites must coincide with those defined for the fish and macroinvertebrates community study (tasks 2D and 2F). In a first approach, inferences about effects to amphibian larvae will be made comparing pH and total metal concentrations recorded on water-column samples, with toxicity values available in the literature. If pH and metal concentrations recorded are above the levels associated with adverse health effects and adults exposure is confirmed, further assessment may be necessary.

Human Population

Human exposure assessment was also planned through the collection of hair samples from the local inhabitants for residue analysis (task 1F) (Figure 3). Human hair samples have long been used to assess heavy-metal exposure from the environment or at the workplace (Jamall and Jaffer 1987; Schuhmacher *et al.* 1991, 1996; Ashraf *et al.* 1994). Hair sample collection is one of the less invasive methods to assess human exposure to heavy metals (Steward and Olson, 1991). The structural and chemical stability of hair, the high trace element concentrations in relation to blood or urine, and the observed correlation between *in situ* environmental pollution and endogenous supply are other advantages pointed out to justify the use of hair samples in monitoring studies (Dörner 1988). Additionally, human hair stores chronological information concerning exposure to substances over a long period of time (Bencze 1990). Washing and digestion procedures of hair samples for atomic absorption spectrometry are well described by several authors (*e.g.*, Sean and Chaudhuri 1996; Štupar and Dolinšek 1996; Hoening and Kersabiec 1996). If human exposure is confirmed, the choice of food items to be analyzed for heavy metal concentrations (task 2A) will be supported by a questionnaire designed to obtain information about age, sex, food ingestion (home grown fruits and vegetables, fish and game animals, breeding cattle, and canned food), source of consumed water, smoking habits, alcohol consumption, health condition and medication, and workplace. Corte do Pinto, Mina de S. Domingos and other South localities inhabitants will be invited to participate in the study and, simultaneously, to answer the questionnaire (task 1F). Comparisons between hair residues recorded in individuals living at different distances from the mine will be performed. Drinking water samples will also be analyzed at the different localities (task 2A). In S. Domingos Mine (SDM) drinking water is obtained directly from Tapada Grande (TG). The values recorded for total concentrations of Cd (0.1 mg/l), Cr (0.15 Mg/l), and Pb (0.36 mg/l), in March 1992, in water-column samples were above the maximum allowed values in surface waters used for human consumption, and of the minimum quality criteria for surface waters (Table 2) (MARN 1998). Visitors and local inhabitants also use this water body for swimming and other aquatic sports.

Child and adult exposure to environmental contaminants through the accidental ingestion of soil cannot be ignored. As was argued by Stanek III *et al.* (1997), although children are more prone to casually or intentionally ingesting soil, adults form the largest proportion of human populations potentially exposed in a contaminated site. Soil ingestion estimates, performed by the same authors, recorded a mean daily soil ingestion, for adults, of 10 mg soil/day, with an upper 95% value of 331 mg soil/day, in a study based on 280 subject/days. Calabrese *et al.* (1997) reported a significantly lower value for children living in a contaminated site (less than 1 mg soil/day) with an upper confidence limit of 160 mg soil/day). This contradictory observation could have resulted from the fact that parents were informed about the contamination of their residential area, and may have changed their behavior. This conclusion reinforces the importance of public information about site specific conditions.

Additionally, although there are probably few individuals potentially exposed through the ingestion of fish and hunting game species, the contamination of these food items will also be considered, because of the popularity of fishing and hunting as recreational activities in this area.

Human exposure through the inhalation of soil fine particles was not considered, because dust emission by rock piles tends to decrease after abandonment, which minimizes environmental air pollution (UN/DTCD and DSE 1992). Moreover, airborne larger particles potentially are eliminated by human nasal hair, which prevents them from passing over the pharynx, and helps to minimize the significance of this exposure pathway (Newman 2000).

Data about total metal concentrations obtained in food items, drinking water, and soil analysis will be used to calculate total daily intakes for humans using the expression proposed by Van De Meent *et al.* (1995). Assumptions about ingestion rates of soil, by children and adults, were described by USEPA (1989). The doses calculated for each element will be compared with toxicity values available in the literature and reported by some databases (*e.g.*, IRIS database provided by USEPA). In a preliminary approach, some inferences can be made about risks for human health, calculating hazard quotients (HQ) for each element and then, assuming additive effects and adding all the HQ in order to calculate a hazard index (HI) (USEPA 1989; Wcislo *et al.* 2002). If this screening assessment indicates a high level of risk, the decision-making process should include the collection of additional confirmatory data and, subsequently, the reformulation of the analysis plan.

CONCLUSION

Based on the management plan designed for the S. Domingos mine area, an analysis plan following a tiered approach was designed in order to perform an integrated risk assessment for this area. The tiered approach helps to prioritize tasks and may allow the scientifically sound basis for eliminating some of them, which is very important when considerable cost and time-consuming tasks are involved.

This primary approach to the S. Domingos mine area risk assessment was mainly based on field biosurveys because of their suitability for the assessment of multiple exposures and effects, especially when these exposures are highly influenced by site-specific factors (Matthiessen 1998; USEPA 1998a). These studies were also mentioned as an appropriate approach to obtain a realistic picture of the impact of metal-polluted sites at the individual, species, and ecosystem levels (Indeherberg *et al.* 1998; USEPA 1998b). Moreover, we believe that information about site-specific effects, yielded by integrated exposures, is more important than that provided by comparing total soil and sediment chemical concentrations with chemical concentrations associated with effects in laboratory tests with single species. These comparisons can overestimate risks because the bioavailability of contaminants is very different from that recorded in the field.

In summary, Tier 1 and Tier 2 tasks constitute the exposure and effects assessment steps of the risk assessment process of the S. Domingos mine area. The data obtained with the planned studies will allow an early screening of risks to human and ecosystem health, following the hazard quotient and the weight of evidence approaches, respectively. Additionally, these data will help risk managers to: (1) establish priorities for remedial action already defined, and only supported by chemical, geological, and hydrological studies; (2) avoid costly and unnecessary clean-up measures; and (3) rethink additional ones.

In the absence of clearly defined regulations and methodologies at the national level, this approach may be an example to be adapted to other contaminated areas of the country, in order to stimulate the development of site-specific risk assessment processes to support their reclamation. Additionally, the integration of human and ecological risk assessments can improve the quality of these reclamation processes because the quality of life of the human populations depends not only on their physical but also on their psychological health, which is greatly influenced by the quality of the surrounding environment and the opportunities that it offers for economical, recreational, and educational activities.

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