# *Erato polymnioides*- a novel Hg hyperaccumulator plant in ecuadorian rainforest acid soils with potential of microbe-associated phytoremediation

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***Highlights (3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

HIGHLIGHTS:

- Hg accumulation capacity was studied in three plant species.
- Hg in acid soil studied is higher than the Ecuadorian threshold (0.1 mg $kg^{-1}$).
- Concentrations of Hg in roots and leaves were higher than in stems.
- All plants showed arbuscular mycorrhizal fungi colonization in their roots.
- Erato polymnioides showed high potential as an Hg hyperaccumulator.

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## Abstract

Mercury (Hg) accumulation capacity was assessed in three plant species (*Axonopus compressus*, *Erato polymnioides*, and *Miconia zamorensis*) that grow on soils polluted by artisanal small-scale gold mines in the Ecuadorian rainforest. Individuals of three species were collected at two sampling zones: i) an intensive zone (IZ, 4.8 mg Hg $kg^{-1}$ of soil) where gold extraction continues to occur, and ii) a natural zone (NZ, 0.19 mg Hg $kg^{-1}$ of soil). In addition, the percentage of arbuscular mycorrhizal fungi (AMF) colonization was determined in plant roots and seven fungal morphotypes isolated from rhizospheric soil. Results suggest a facilitation role of native and pollution adapted AMF on Hg phytoaccumulation. E.g., *E. polymnioides* increased Hg accumulation when growing with greater AMF colonization. We concluded that *E. polymnioides* is a good candidate for the design of microbe-assisted strategies for Hg remediation at gold mining areas. The consortia between *E. polymnioides* and the AMF isolated in this study could be instrumental to get a deeper understanding of the AMF role in Hg phytoaccumulation.

**Keywords**: *Heavy metals; bioremediation; pollution; artisanal scale gold mining; arbuscular mycorrhizal fungi; southern Ecuador*

## 1. Introduction

Gold (Au) price has tripled in the last 10 years and artisanal small-scale gold mining (ASGM) has increased substantially in rural areas all over the world (García et al., 2015; Veiga et al., 2014). Around 16 million people are directly involved in this activity nowadays, producing 380-450 tons of gold annually. In many cases, the process used in ASGM for Au extraction is mercury (Hg) amalgamation, that consists in grinding the

mineral (raw material) and mixing it with Hg (Seccatore et al., 2014). During amalgamation, an Au-Hg alloy is produced and heated in open vessels to separate both metals from undesired matter. Pure Au and volatilized Hg are then obtained.

Hg is one of the most toxic heavy metals and a global pollutant that biomagnifies through the food chain, posing a threat to human and animal health (Navarro et al., 2009; Pirrone et al., 2009). ASGM using amalgamation is estimated to release into the environment 37% of global air emissions of Hg and 1400 tons $year^{-1}$ of Hg directly to the soil and water sheds (Gibb and O'Leary, 2014). It is also responsible for Hg emissions that lead to massive aerial Hg contamination and pollution of terrestrial ecosystems through rainfall deposition (727 tons $year^{-1}$) not only at ASMG sites but also at other areas (Seccatore et al., 2014; United Nations Environment Programme, 2013). Anomalously high concentrations of Hg (over 4 mg Hg $kg^{-1}$ soil) are reported by several studies wherever the Hg amalgamation process is practiced (García et al., 2015; Terán-Mita et al., 2013). However, volatilization of Hg from soil and water occurs and Hg returns to the atmosphere generating a cycle, with volatilization from soil being more relevant than from the oceans (Bjerregaard and Andersen, 2007). Plants contribute to volatilization of Hg by uptaking Hg from soil and releasing it into the atmosphere through stomata in a process called phytovolatilization (Ali et al., 2013).

Communities living nearby and downstream ASGM are exposed to Hg vapor, and regularly consume food heavily contaminated with methyl mercury (MeHg) (Gibb and O'Leary, 2014). Elemental Hg and MeHg are toxic to the nervous, digestive, and immune system. They can cause mental retardation, seizures, vision and hearing loss, delayed development, language disorders, and memory loss, being fatal in some cases (World Health Organization, 2006). Reducing the impacts of ASGM on human health and the environment has become a major concern for society and many governments worldwide. Thus, reducing the use of Hg in ASGM was included in the Minamata Convention on Hg, signed in 2013 in Minamanta, Japan (United Nations Environment Programme, 2013).

Several techniques to encapsulate and stabilize Hg are being researched to reduce the impact of Hg emissions at ASGM sites, such as bioremediation and, especially, phytoremediation (Mani and Kumar, 2014). Hg phytoremediation with so called metal hyperaccumulator plants has attracted interest as an inexpensive, low-impact and visually benign technique compared to traditional physical approaches (Lorestani et al., 2012).

Historically, the term "hyperaccumulator" has undergone significant changes since it was defined by Brooks et al. (1977). The first definition was "plants with Ni concentrations higher than 1000 mg $kg^{-1}$ dry weigh". Later, Baker and Brooks (1989) extended the concept to more metals and defined hyperaccumulators as plant species which accumulate greater than 100 mg kg-1 dry weight Cd, or greater than 1000 mg kg-1 dry weight Ni, Cu and Pb or

greater than 10000 mg kg-1 dry weight Zn and Mn in their shoots. However, other authors suggest that criteria are unnecessarily conservative (van der Ent et al., 2013). Nowadays, hyperaccumulators are defined as plants that achieve 100-fold higher shoot metal concentration, compared to crop plants or common non-accumulator plants (Barceló and Poschenrieder, 2003; Chamba et al., 2016; Redondo-Gómez, 2013). Thus, they have tremendous potential for remediation of metals in the environment.

Many plant species have been cataloged as Hg hyperaccumulators (Angle et al., 2001; Lorestani et al., 2012), e.g. *Jatropha curcas* and *Piper marginathum* from tropical rainforests and *Rumex induratus* and *Marrubium vulgare* from the Iberian Peninsula. *P. marginathum* reached Hg tissue concentrations in the range of 0.53-6 mg $kg^{-1}$ (Marrugo-Negrete et al., 2016) and those from the Iberian Peninsula showed 8.3-67.2 mg $kg^{-1}$ of Hg in their roots (Moreno-Jiménez et al., 2006). Prasad and De Oliveira (2003) found Asteraceae family members as one of the best hyperaccumulator plant species and a good candidate for phytoremediation compared to several organism, including mycorrhizal and non-mycorrhizal fungi, about 400 hyperaccumulating agricultural, vegetable crops and ornamentals plants (Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphobiaceae).

Finding native hyperaccumulator plants to the ASGM areas has gained attention during recent years. Native metal hyperaccumulator plants are harmless to native biodiversity, require less handling and acclimatization and are also, in most cases, highly tolerant to the contaminants that need to be removed (Sarma, 2011). However, native hyperaccumulator plants are difficult to cultivate sometimes, and may have low growth rates and biomass, lowering the metal uptake (Goltapeh et al., 2013). In this sense, mycorrhizal fungi live associated to most of the higher plants in different forms, with arbuscular mycorrhizal fungi (AMF) associations with the roots of terrestrial plants being the most widespread. AMF modify the bioavailability and mobility of toxic metals in the soil and facilitate metal uptake by the plants as well as increase the host plants biomass production in polluted ecosystems (Leung et al., 2013; Teixeira et al., 2014). AMF constitute a bridge for nutrient and heavy metal transport from soils to plant roots, such as N, P, K, Ca, S, Zn, Co, Ni and Cu through extensive hyphal network. They also bind metals in the hyphae outside the root, preventing metals to move to the aerial parts of the plant and, in the case of Hg, preventing volatilization too (Sarwar et al., 2017). Then, native AMF have arisen as promising alternative and as an innovative tool to replace or supplement present treatment processes in order to assist phytoremediation.

This research has two main objectives. Firstly, to assess the potential of three native plants as Hg hyperaccumulators in Chinapintza (Zamora-Chinchipe province, southeast Ecuador),

a site with a long ASGM tradition. Secondly, to evaluate AMF colonization in the plant species and the spores of the most prominent AMF isolated.

## 2. Materials and Methods

### *2.1.Study area*

As shown in Figure 1, the study area is located in Chinapintza (Zamora-Chinchipe Province, southeast Ecuador), beside the Condor mountain range, near to the Peruvian border (at 1854 masl; 4°02'16.6"S 78°34'14.9"W). Zamora-Chinchipe Province is well known by its ASGM activity: around 23% (282.998 ha) of the total surface is dedicated to ASGM (Sacher and Báez, 2011). The study area was divided into two zones: (1) a natural zone (NZ) free of influence of ASGM and (2) an intensive zone (IZ) highly deteriorated by ASGM that continues to occur.

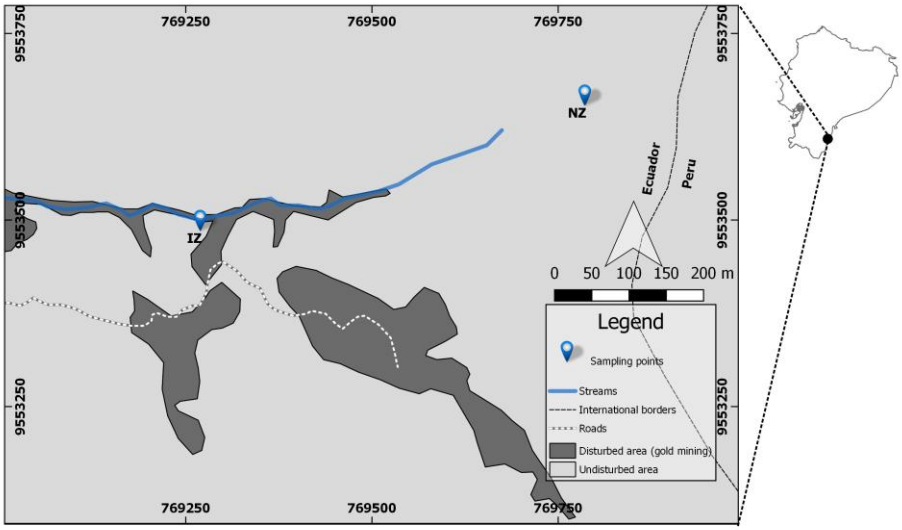



**Figure 1.** Map of the sampling points.

### *2.2.Plant and soil sampling and processing*

Three abundant plants present in NZ and IZ were selected as potential Hg hyperaccumulators: *Axonopus compressus* (SW.) P. Beauv (Poaceae), *Erato polymnioides* DC. (Asteraceae), and *Miconia zamorensis* Gleason (Melastomataceae). Seven individuals of each species and their rhizosphere soil were taken as samples in each of the two zones, i.e. a total of 42 individuals and its surrounding soil.

According to the recommendations of Tack and Verloo (1996), sampling took place far from active roads and the surface of the fresh plant material was checked to be free of dust. In the laboratory, plant samples were washed with ultra-pure water (Merck-Millipore Milli-Q), placed into paper bags and dried in an oven at 60°C for one week. Soil samples (0.5-1.0 kg each) were dried at 60°C until constant weight. Dried samples (plant tissues and soil) were weighed and mechanically grounded using a stainless steel grinder (particle diameter

100 μm) for digestion. In the case of *A. compressus*, experiments were carried out only in roots and leaves, since it lacks of a real stem. *A. compressus* shows a pseudo stem formed by tightly packed overlapping leaf sheaths, like in other members of *Poaceae* family.

*2.3.Soil physicochemical parameters*

Soil pH was determined on a soil:water mixture (1:2.5) by a potentiometric method (Jaworska et al., 2016). Soil organic matter (SOM) was determined by the Walkley & Black method (Rial et al., 2016; Walkley and Black, 1934). Available phosphorus (P), inorganic nitrogen ($NH_4$) and potassium (K), were extracted in an Olsen modified extract pH 8.5 (Olsen and Sommers, 1982), and quantified by photocolorimetry-blue phosphomolybdate (P), photocolorimetry-blue indofenol ($NH_4$), and atomic absorption spectroscopy (AAS). Cationic exchange capacity (CEC) was measured in ammonium acetate buffered at pH 7 with barium chloride, and finally soil texture was determined by the method of Bouyucos (Day, 1982). All measurements were performed on seven replicates.

*2.4.Hg quantification in soil and plant samples*

Dried samples were weighed, 0.2 g of each plant tissue (roots, stem and leaves) per individual and 1 g of rhizosphere soil, and were left to soak in aqua regia, i.e. a mixture of HCl and $HNO_3$ in a 3:1 ratio (v/v), for a week. Next, they were digested on an open heat block (environmental express 54 Hot block SC154) for 2 h. After cooling, the samples were diluted to 100 ml with HCl 0.1 M and stored until metal determination. Total Hg concentration was determined following the hydride-generation technique in an atomic absorption spectrophotometer (Perkin-Elmer, AANALYST-400). A Hg standard calibration curve (100, 200, and 300 $\mu g l^{-1}$) was prepared in 10 ml of an acid mixture containing 1.5% $HNO_3$ by triplicates. Two blank samples were also run simultaneously to estimate background metal contamination from the digestion procedure. For each sample, 10 ml of acid mixture containing 1.5% $HNO_3$ were added to 5 mL of the digestion mixture (prepared by triplicates). Hg was determined using an aqueous solution of 3% (w/v) $NaBH_4$ in a 1% (w/v) NaOH solution freshly prepared and filtered as reducing agent. An electrodeless discharge lamp was used (Olmedo et al., 2013). Analytical grade chemical reagents and highly purified deionized water were always used.

*2.5.Hg phytoextraction capacity calculation*

To evaluate the Hg phytoextraction capacity of selected plant species, the bioaccumulation factor (BF) was calculated as the ratio between the Hg concentrations in 3 plant tissues (root, stem, leaves) and those in its corresponding rhizosphere soil sample: $BF=C_{tissue}/C_{soil}$ (González and González-Chávez, 2006). $C_{tissue}$ and $C_{soil}$ are Hg concentrations (mg Hg $kg^{-1}$ of dry weight). To evaluate the plants ability to transfer Hg from soil to their aerial parts, the translocation factor (TF) was calculated as the ratio between Hg concentrations in aerial plant parts (leaves and stem) and those in the plant root: $TF= C_{aerial}/C_{roots}$ (Chopin et al.,

2008; Conesa et al., 2006). $C_{aerial}$ and $C_{roots}$ are Hg concentrations (mg Hg $kg^{-1}$ of dry weight).

*2.6.Quantification of AMF colonization and spores morphotypes*

Root samples from the three species were collected at the NZ and the IZ to determine the presence of AMF colonization ($\%AMF_{col}$). Roots were washed thoroughly with running water to remove organic debris and soil particles and cut into small segments of 1 cm in length. AMF colonization was assessed in each root segment following the method of (Giovannetti and Mosse, 1980). Briefly, root segments were stained with 0.05% tryphan blue in lactophenol, and then washed with clear lactophenol to remove the excess of the colorant. A total of 25 stained root segments were randomly selected for each plant species and mounted on microscopic slides (five segments per slide). AMF colonization was then assessed microscopically based on the presence of a blue stain. The percentage of root colonization was calculated per plant species using the ratio of effectively stained segments (presence of colonization) to the total of microscopically analyzed root segments for the particular species per 100. Measurements were performed for three replicates of each plant species in two sampling zones.

AMF morphotypes associated with the roots of *E. polymnioides* and *M. zamorensis* were isolated through the establishment of trap cultures. Five rizospheric soil samples per plant species were placed in 2 l pots planted with a highly colonizable plant host (*Avena sativa* L.) during five months. To fill each pot, fresh rizospheric soil samples were 1:1 mixed with sterilized quarzitic sand (steam-sterilizered sand at 100°C forth periods of two hours each). After planting, *A. sativa* plants were subjected to a regime of irrigation suspension for two weeks to further promote AMF sporulation (Morton et al., 1995). AMF spores that occurred in trap pots after five months were isolated by sieving 50 g of soil followed by centrifugation in sucrose, as recommended by Sieverding et al. (1991). Pools of spores sharing the same morphology were created under a stereomicroscope (40x) and further separated into morphotypes by color and size. Photographs of each morphotype were obtained from permanent microscope slides prepared using polyvinyl alcohol-lactic acid-glycerin (PVLG) (Koske and Tessier, 1983) as mounting media under a compound microscope. Morphotypes were morphologically described based on the number, size and ornamentation of spore walls, reaction to Melzer reagent (iodine-potassium iodide-chloral hydrate), and other criteria such as type of hyphal attachments and cicatrix if present. Taxonomic identification down to genera level and to species level when possible was performed according to an on-line AMF species catalogue and descriptions available at the International Culture Collection of Mycorrhizal Fungi web site (http://invam.caf.wvu.edu/index.html) following the classification given by some authors (Redecker et al., 2013).

*2.7.Statistical analysis*

SPSS version 20 software was used to carry out t-tests and ANOVA tests. The t test was used to determine whether there were significant differences between two means corresponding to two groups. For example, t test was used to elucidate if there were significant differences between both zones in soil physicochemical parameters. The ANOVA test was used to establish significant differences between more than two means, corresponding to several groups. It allows drawing conclusions about Hg concentrations in plant tissues, bioaccumulation and translocation factors and AMF colonization and richness.

## 3. Results

*3.1.Soil physicochemical parameters and Hg concentrations*

Acidic pH, high Fe and low N, P and K concentrations were recorded in all soil samples from both the NZ and the IZ (Table 1). There were small differences between soils from the sampling zones on most of the assayed parameters with the exception of N, and as expected, Hg concentrations ($p<0.05$). N and Hg were found at higher concentrations in soil samples from the IZ.

**Table 1.** Soil parameters from the sampling zones. NZ: natural mining zone; IZ: intensive mining zone. Values are expressed as mean of seven independent measurements ± standard deviation

| Soil parameter | Sampling zone | |
|---|---|---|
| | NZ | IZ |
| pH ($H_2O$) | 4.3 ± 0.5 | 4.1 ± 0.5 |
| SOM (%) | 2.8 ± 0.9 | 2.4 ± 0.5 |
| N (mg $kg^{-1}$) | 44.8 ± 6.5 | 85.3 ± 10.5 |
| P (mg $kg^{-1}$) | 6.9 ± 0.9 | 8.6 ± 2.3 |
| K (cmol $kg^{-1}$) | 0.14 ± 0.03 | 0.12 ± 0.07 |
| CEC (cmol $kg^{-1}$) | 6.3 ± 1.4 | 6.9 ± 1.2 |
| Fe (mg $kg^{-1}$) | 505 ± 154 | 664 ± 207 |
| Hg (mg $kg^{-1}$) | 0.19 ± 0.09 | 4.8± 1.2 |
| S (mg kg-1) | 33.2 ± 3.9 | 123.1 ± 41.5 |

As it can be seen in Table 1, sulfur is an important component of the acidic soils we studied. Sulfur concentration in the IZ is almost four times that of the NZ. The substantial increase of sulfur in the IZ compared to the NZ reflects the gold mining practices in the IZ. In addition, it is important to discuss the potential implications of high sulfur contents for

Hg phytoremediation. Moreno et al. (2004) reported that sulfur in soil stimulates the accumulation of Hg in plant tissues. For example, *Brassica juncea* can concentrate Hg to 40 mg $kg^{-1}$ in the plant canopy tissue after application of ammonium thiosulfate on mine waste contaminated with 2.8 mg Hg $kg^{-1}$. In fact, sulfur-mediated stimulation of Hg bioaccumulation has been reported by several researchers (Boudou et al., 1991; Moreno et al., 2005; Muddarisna et al., 2013; Wang et al., 2014).

### *3.2.Hg concentrations in plants tissues*

Hg concentrations in tissues (root, stem and leaves, for *E. polymnioides* and *M. zamorensis* and stem and root for *A. compressus*) from the studied species are shown in Figure 2. The highest values of Hg in roots and leaves of *E. polymnioides* and *M. zamorensis* were recorded in the IZ compared to the NZ as expected ($p<0.05$). Only in the stems the differences were not significant ($p>0.05$). Surprisingly, concentrations of Hg in the tissues of *A. compressus* were not statistically significantly different in both zones ($p>0.05$).

The highest averages for Hg accumulation in roots and leaves were recorded in *E. polymnioides* individuals from the IZ (3.56 mg $kg^{-1}$and 1.48 mg $kg^{-1}$ respectively), followed by *M. zamorensis* individuals from the IZ (2.06 mg $kg^{-1}$and 0.98 mg $kg^{-1}$ respectively). Considering the IZ, the leaves of *E. polymnioides* showed a significant difference compared to *A. compressus* ($p<0.05$) but unsignificant to *M. zamorensis* ($p>0.05$). In the case of roots, concentrations of Hg in *E. polymnioides* were significantly different than concentrations in roots of both plants ($p<0.05$). This fact can ascribes a superior potential to *E. polymnioides* than *M. zamorensis* and *A. compressus* to accumulate Hg in soils with high Hg concentrations.

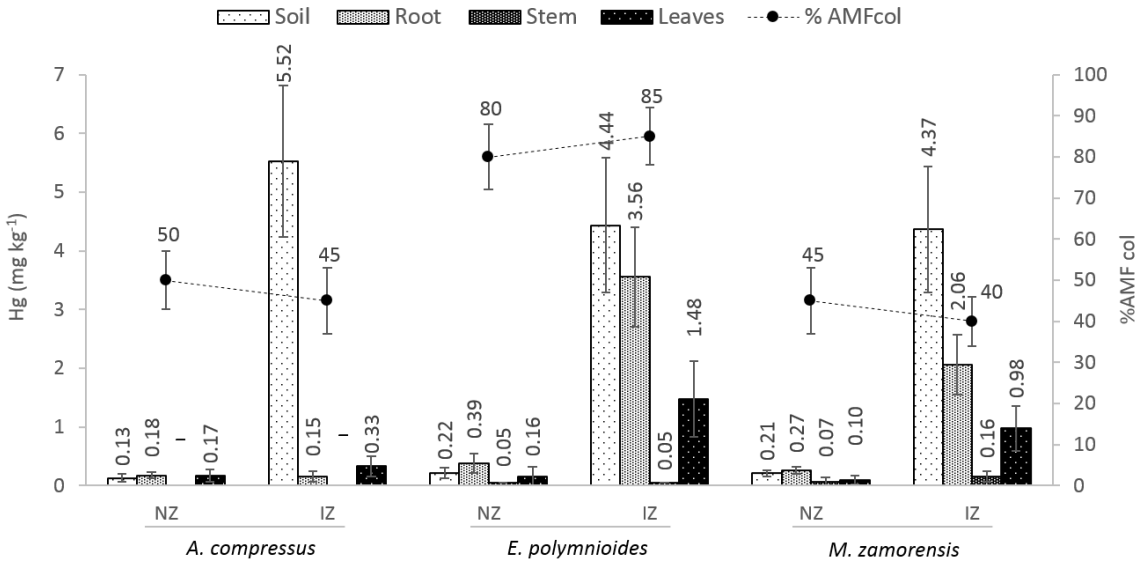



**Figure 2.** Hg concentrations (mg Hg $kg^{-1}$ of dry weight) in plants tissues (root, stem and leaves), and percentage of root arbuscular mycorrhizal fungal colonization ($\%AMF_{col}$) on

individuals of each of the studied species collected at the natural mining zone (NZ) and the intensive mining zone (IZ).Values are expressed as mean of seven independent measurements ± standard deviation.

### *3.3.Bioaccumulation and translocation factors*

Bioaccumulation factors were generally higher in the NZ thanks to the lower Hg concentrations in the soil. Focusing on the IZ, roots seemed to be the preferred tissue for Hg bioaccumulation, since they concentrated the top bioaccumulation factors of all tissues on two of the three species studied. The highest bioaccumulation factor corresponded to the roots of *E. polymnioides* (0.80) even though the concentration of Hg in the soil was as high as 4.4 mg $kg^{-1}$.

A part of Hg looked to be further pumped up to the leaves in *E. polymnioides* and *M. zamorensis* (Figure 3) according to leaves' translocation factors over 0.3. Nevertheless, *A. compressus* translocated a higher proportion of Hg from the roots to the leaves and it accounted for the largest leaves/root translocation factor.

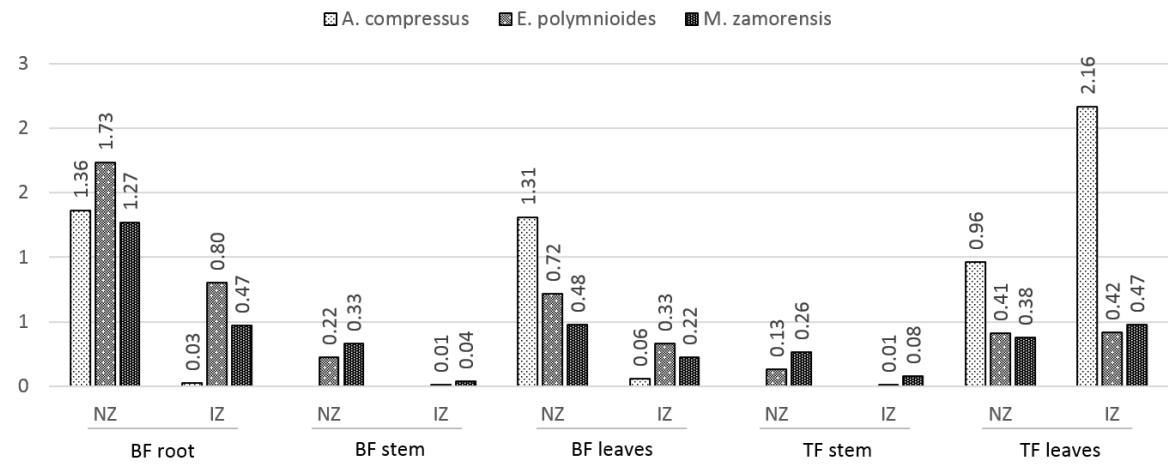


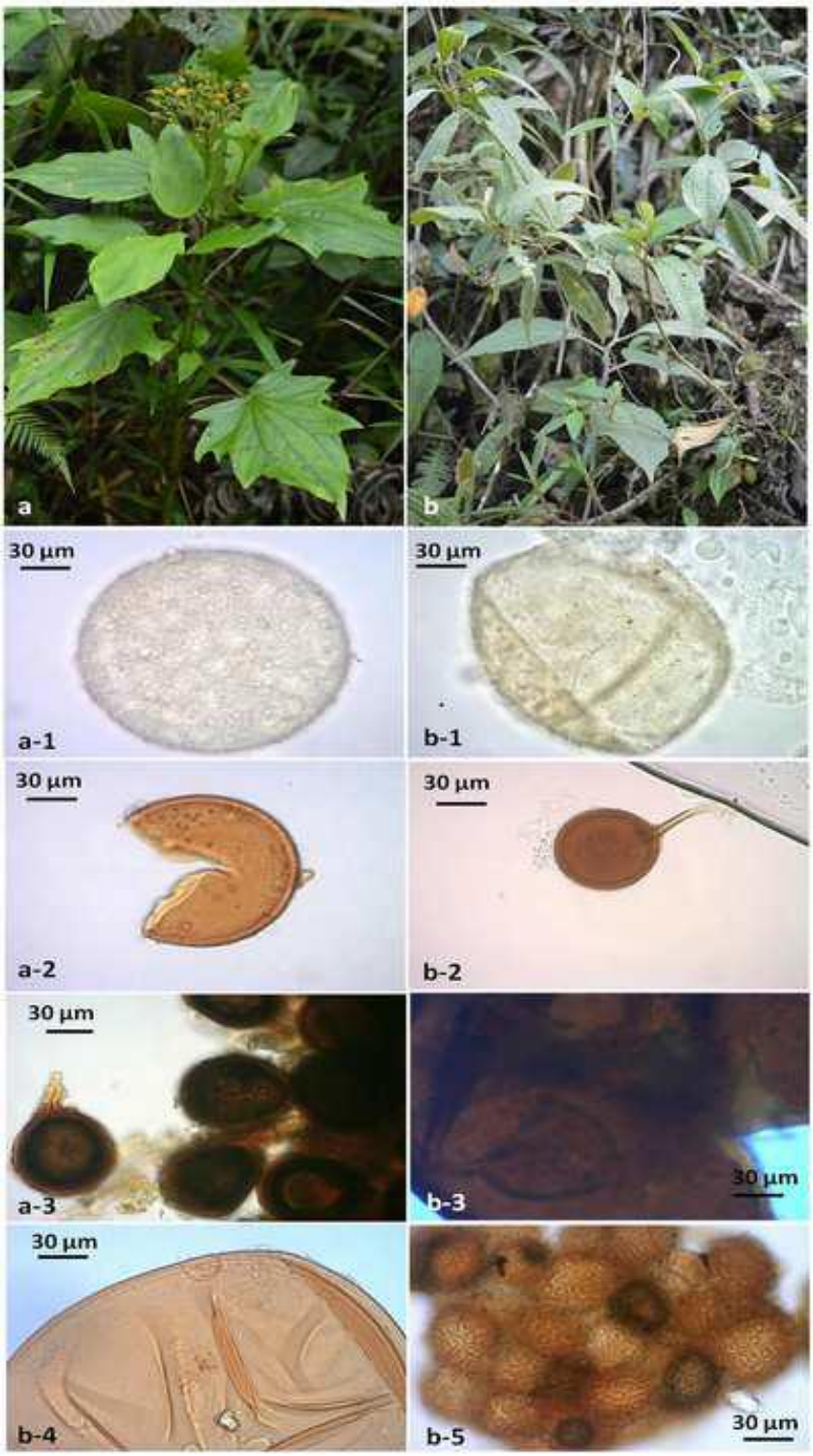

**Figure 3.** Bioaccumulation factor (ratio of the plant tissue to soil metal concentration, BF) and translocation factor (ratio of the stem and leaves to root metal concentration, TF) for Hg in three native plants from natural mining zone (NZ) and intensive mining zone (IZ). Values are calculated based on the corresponding mean values in Figure 2.

### *3.4.AMF colonization and richness analyses*

All plants showed AMF colonization in their roots (see $\%AMF_{col}$ in Figure 2). *E. polymnioides* showed by far the highest root colonization by AMF ($p<0.05$). Interestingly, root colonization in *E. polymnioides* was higher in the IZ than in the NZ. The opposite was observed in the other two species. Since *A. compressus* showed the lowest values of Hg accumulation (Figure 2) and mycorrhizal colonization, the analysis of AMF richness was circumscribed to *E. polymnioides* and *M. zamorensis*. Seven morphotypes from the

following genera were identified in AMF morphotype based characterization: *Glomus*, *Acaulospora*, *Ambispora* and *Racocetra* (Figure 4). Three of these morphotypes (*Ambispora* sp., *Glomus* sp. 1 and *Glomus* sp. 2) were found associated to *E. polymnioides* while five morphotypes (*Ambispora* sp., *Glomus* sp. 3, *Racocetra* cf. *gregaria*, *Acaulospora* cf. *capsicula* and *Sclerocystis sinuosa*) were associated to *M. zamorensis*. Only one morphotype (*Ambispora* sp.) was found in common between both plant species.

**Figure 4. Arbuscular mycorrhizal fungi morphotypes associated with the rhizospheric soil of a)** *Erato polymnioides* (morphotypes a-1, a-2, a-3) **and b)** *Miconia zamorensis* (morphotypes b-1, b-2, b-3, b-4, b-5). **a-1)** *Ambispora* sp. **a-2)** *Glomus* sp. 1, **a-3)** *Glomus* sp. 2, **b-1)** *Ambispora* sp.**b-2)** *Glomus* sp. 3, **b-3)** *Racocetra* cf. *gregaria* (sporogenous cell) **b-4)** *Acaulospora* cf. *capsicula* (cicatrix), **b-5)** *Sclerocystis sinuosa*.



## 4. Discussion

Chemical properties observed in Chinapintza soil samples were those typical of tropical humid forest soils: acidic pH and low P, N and K concentrations. These results are in agreement with other analyses conducted in the region (Jiménez et al., 2007). Hg concentrations recorded at both sampling zones (NZ: 0.19 mg $kg^{-1}$; IZ: 4.8 mg $kg^{-1}$) exceed the level reported for regular crustal abundance (0.01-0.05 mg $kg^{-1}$) (Rudnick and Gao, 2003), and the threshold for polluted soils in Ecuador (0.1 mg $kg^{-1}$) (Ecuadorian Ministry of the Environment, 2015). Hg concentration in the IZ is similar to the upper-end value reported at ASGM areas in Bolivia (0.5-2.4 mg $kg^{-1}$) (Terán-Mita et al., 2013). However, Hg in the IZ is far lower than polluted mining sites in Asia, where Hg reaches 44 mg $kg^{-1}$ (Li et al., 2013) and 35 mg $kg^{-1}$ (Zhang et al., 2010) in China.

Soil pHs, electric conductivity and sulphur in polluted soils showed influence on plant growth and correlation with phytoremediation in previous studies (Adamczyk-Szabela et al., 2015; Salimi et al., 2012). High electric conductivity levels of irrigation water can affect to the absortion of metals by the plant (Salimi et al., 2012). On the other hand, when the pH is lower the metal ions show greater cation exchange capacity and become more available in the aqueous medium, making the metal more bioavailable to plants, as demonstrated in *Valeriana officinalis* (Adamczyk-Szabela et al., 2015). The most intensive germination in *Valeriana officinalis* and plant growth was observed at pH=5.1. Increased alkalinity to pH=10 significantly inhibited both the seed germination and plant growth. Valerian seeds inoculated in soil characterized by a low or very high pH values (3.5 and 13.0) failed to germinate even in non-contaminated soils, showing the negative influence of too low or too high pH value.

Soil Hg concentration in the NZ remains higher than the Ecuadorian nation-wide reference value of 0.1 mg $kg^{-1}$, highlighting the slow mobility of Hg in terrestrial ecosystems (Hintelmann et al., 2002) and the need of effective extraction methods. Slow mobility of Hg in polluted soils is thought to be a consequence of the presence of no readably bioavailable chemical species, e.g. Hg-sulfide and elemental Hg (Piani et al., 2013). Therefore, it is not accumulated in plant tissues in significant amounts. Hg accumulation relies on plants ability to absorb and transport the Hg−thiosulfate complex (Wang et al., 2012). The ability to convert Hg-sulfide into Hg-thiosulfate has been reported for a number of sulfur-oxidizing bacteria (Vázquez-Rodríguez et al., 2015), which are responsible for making Hg bioavailable for plants. However, Hg-sulfide oxidation and plant Hg-thiosulfate uptake are not necessarily coupled, since the first process might occur far from plant roots. AMF associated with plant roots may improve plant uptake of bioavailable forms of Hg (Ekamawanti et al., 2014; Pichardo et al., 2012; Selvaraj et al., 2005). In soils like those in Chinapintza, in which phosphorous concentration is relatively low, AMF colonization is expected to be favoured since it brings several benefits for plants to fulfilling nutrient requirements (Ryan et al., 2000). Altogether, these observations could explain why the

results of this study suggest a relationship between the amount of AMF colonization and the species' ability to accumulate Hg in their tissues. *E. polymnioides* showed the highest Hg accumulation capacity as well as the highest percentage of AMF colonization. High percentages of AMF colonization in heavy metal hyperaccumulating plants of the Asteraceae family (the one to which *E. polymnioides* belong) have been reported (Turnau and Mesjasz-Przybylowicz, 2003).

In fact, *E. polymnioides* is the only species in this study that showed no saturation in its Hg accumulation capacity when growing in the IZ. Furthermore, it was the only species displaying an increase in AMF colonization in the IZ compared to the NZ. Both *A. compressus* and *M. zamorensis* showed clear signs of Hg accumulation capacity saturation when growing at concentrations of Hg as high as 4.8 mg $kg^{-1}$ in the IZ and a reduction of AMF colonization compared to the NZ. Hg accumulation capacity saturation has been reported in other plant species, such as *Jatropha curcas* (Euphorbiaceae family), at Hg concentrations in the range of those in the IZ (Marrugo-Negrete et al., 2015). Asteraceae family shows advantages, such as Hg tolerance and efficiency to accumulate Hg in the polluted soil as it behaves as a hyperaccumulator. Also, it was identified as the most common species in the study sites showing Hg as the major metal distribution in the soil.

Interestingly, only three AMF morphotypes were found associated to *E. polymnioides*. This might suggest that Hg accumulation is independent of AMF diversity. Instead, Hg accumulation could be based on a core of highly infective strains of AMF capable of enhancing plant nutritional status and making Hg-bioavailable species accessible to plant tissues as a side effect. A recent study has demonstrated that native AMF adapted to soil polluted conditions can effectively increase the total content of metals present in plant tissues as well as plant root biomass production (Ouaryi et al., 2016). On the other hand, the involvement of AMF in phytoremediation may also occur through specific mechanisms of metal retention and immobilization in the mycelium of the fungus, reducing its translocation to the stem (Cabral et al., 2015; Miransari, 2011). The production of metallotioneins by AMF increases the deposition of heavy metals in their cells (Miransari, 2011). In this sense, this symbiosis protects the plant due to the stabilization of metals in the radical compartment, which is known as phytostabilization mediated by microorganisms (Meier et al., 2012). AMF-mediated phytostabilization has also been proposed as an explanation for the decrease in Hg phytovolatilization in mycorrhizal plants compared to non-mycorrhizal plants (Yu et al., 2010).

Values of Hg found in roots of *E. polymnioides* individuals from the IZ (3.56 mg $kg^{-1}$) represent as much as the 74% relative to rhizosphere soil Hg concentration in the IZ. These values are comparable to those reported for other members of the Asteraceae family: *Matricaria recutita* (1.57 mg $kg^{-1}$ in aerial tissue) and *Scolymus hispanicus* (2.72 mg $kg^{-1}$), growing in polluted soils with 5.53 mg $kg^{-1}$ of Hg (Millán et al., 2006). Considering that

0.5-1.0 mg Hg $kg^{-1}$ in plant tissues can produce growth depression (Kabata-Pendias, 2011), *E. polymnioides* showed an elevated tolerance to this heavy metal. In addition, *E. polymnioides* displayed the least root-to-leaves Hg translocation, in contrast to *A. compressus*, which has the ability to translocate an important fraction of the accumulated Hg from the roots to its leaves, especially at the IZ (2.16). Based on these findings, *E. polymnioides* could be seen mainly as a Hg stabilizer species while *A. compressus* capable of phythoextracting Hg (incorporation and redistribution to the aerial parts of the plant).

Regardless the final goal of a remediation strategy, heavy metal phytoextraction is desirable in stages where appropriately cutting and final disposal of the aerial plant biomass has been previously covered. In contrast, phytostabilization could be more favorable in less accessible locations, where plant roots and rhizospheric microbial interactions can inmobilize Hg, reducing its incorporation into ground and/or water bodies and food chain (Sarma, 2011). In a geographical setting like that of Chinapintza, with limited accessibility, highly dense tropical forests and many ASGM sites around a water stream that goes directly into downstream communities, Hg phytostabilization is preferred over phytoextraction. A highly efficient accumulator and tolerant plant such as *E. polymnioides* could represent an attractive strategy for the environmental remediation of Chinapintza soils affected by ASGM. Even more, considering it is native to the area, an increase in its population size for remediation purposes should not represent an ecological treat

On the other hand, volatilization of $Hg^0$ by transpiration is a possible route for Hg detoxification in plants (Moreno et al., 2008). Future studies are essential to evaluate the phytovolatilization of Hg and the possible contribution to air pollution of this native plant.

## 5. Conclusions

*E. polymnioides* (Asteraceae family) showed high potential as an Hg hyperaccumulator plant with higher affinity of mycorrhizal association helpful to survive against metal toxicity. Thus, it is a suitable candidate to be considered as an Hg hyperaccumulator when designing environmental friendly and sustainable strategies for Hg remediation at ASGM areas. A deeper understanding of AMF role in Hg phytoextraction should be paid a special attention in future studies. The availability of *E. polymnioides*, together with the associated AMF diversity isolated in the present study, could be instrumental for this task.

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**Figure**

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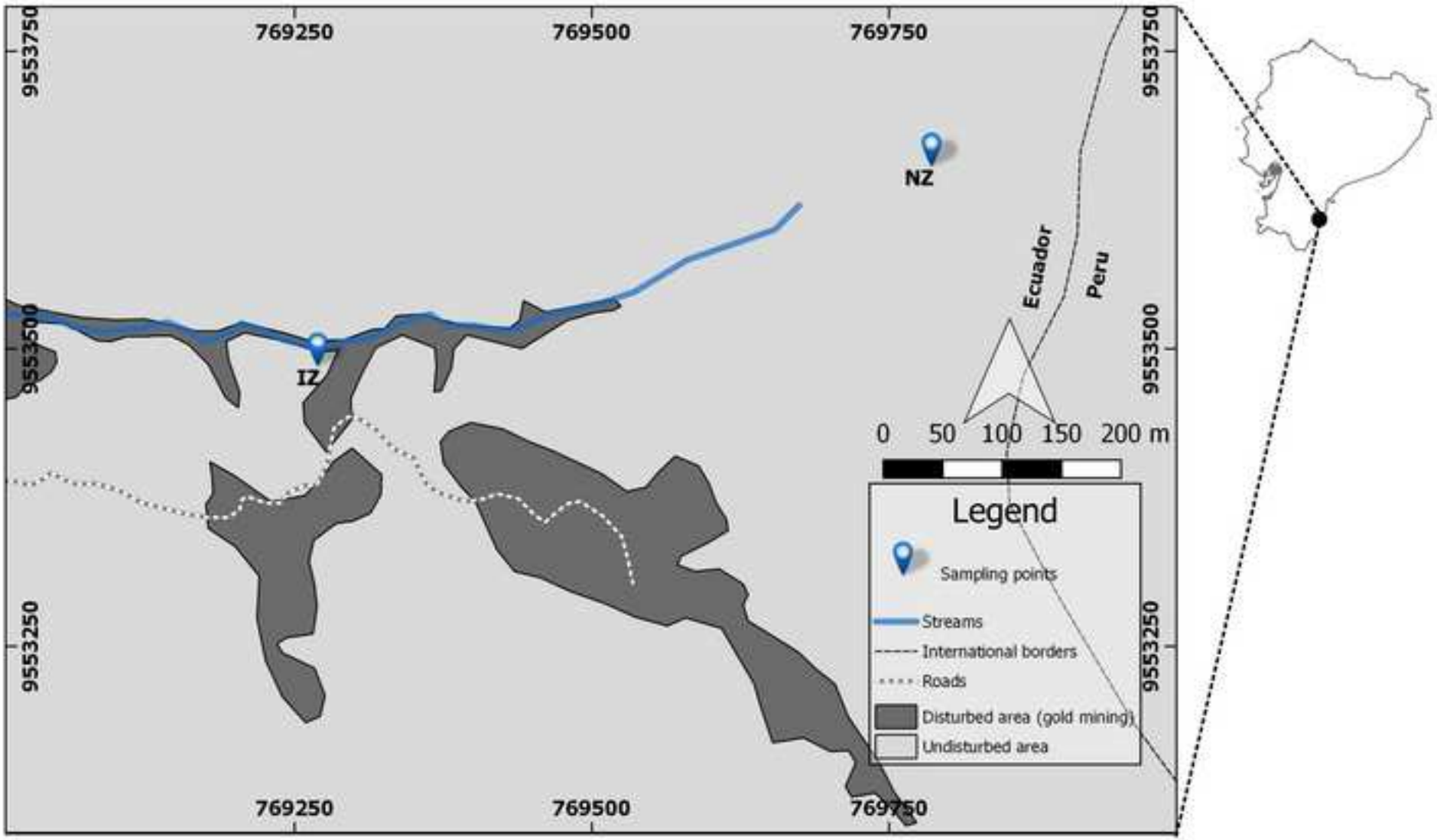

Figure2

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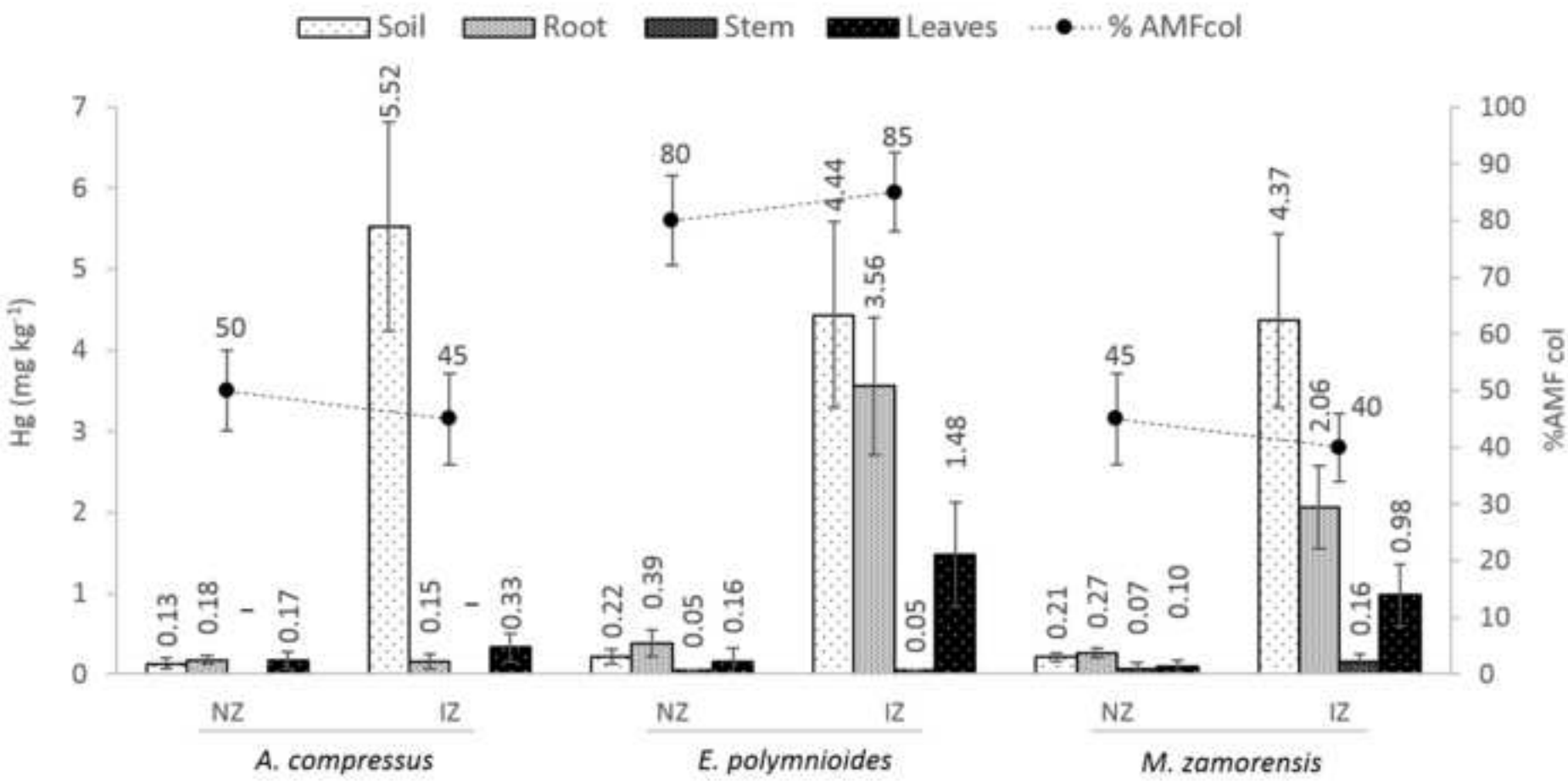

Figure3

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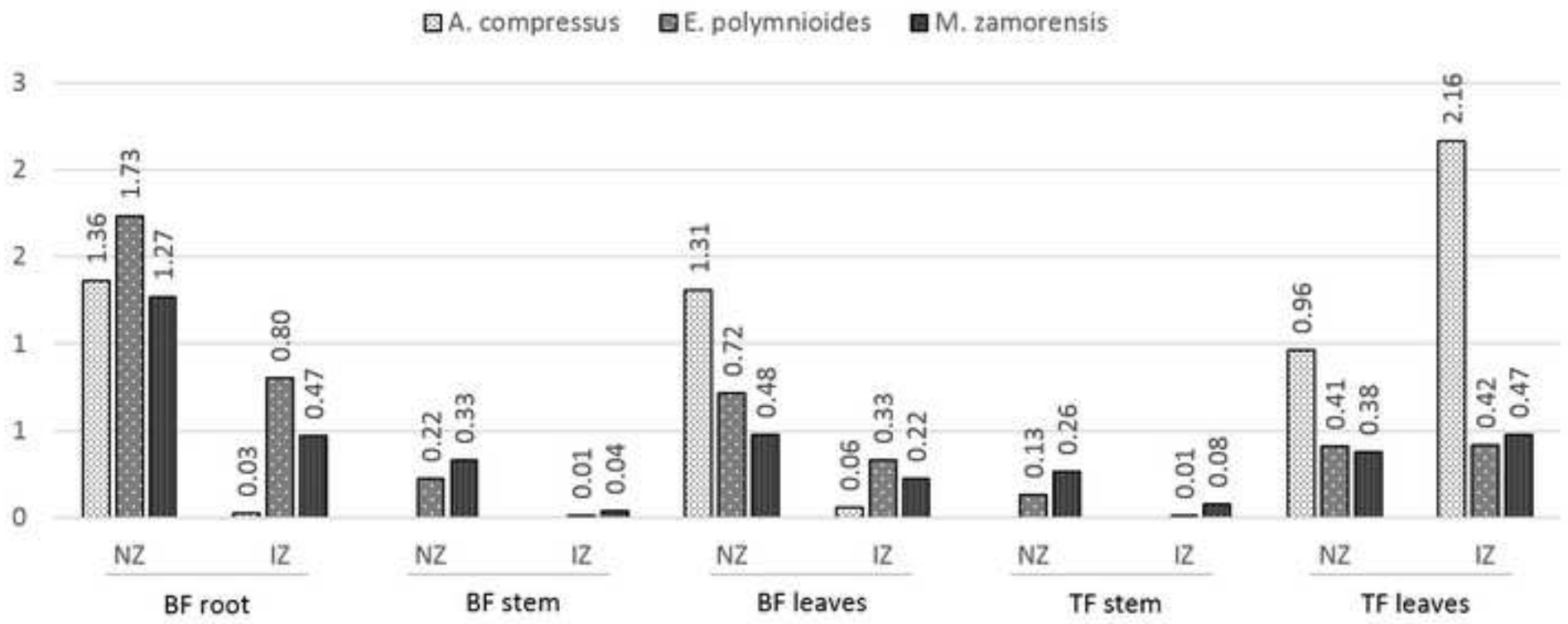

Figure4

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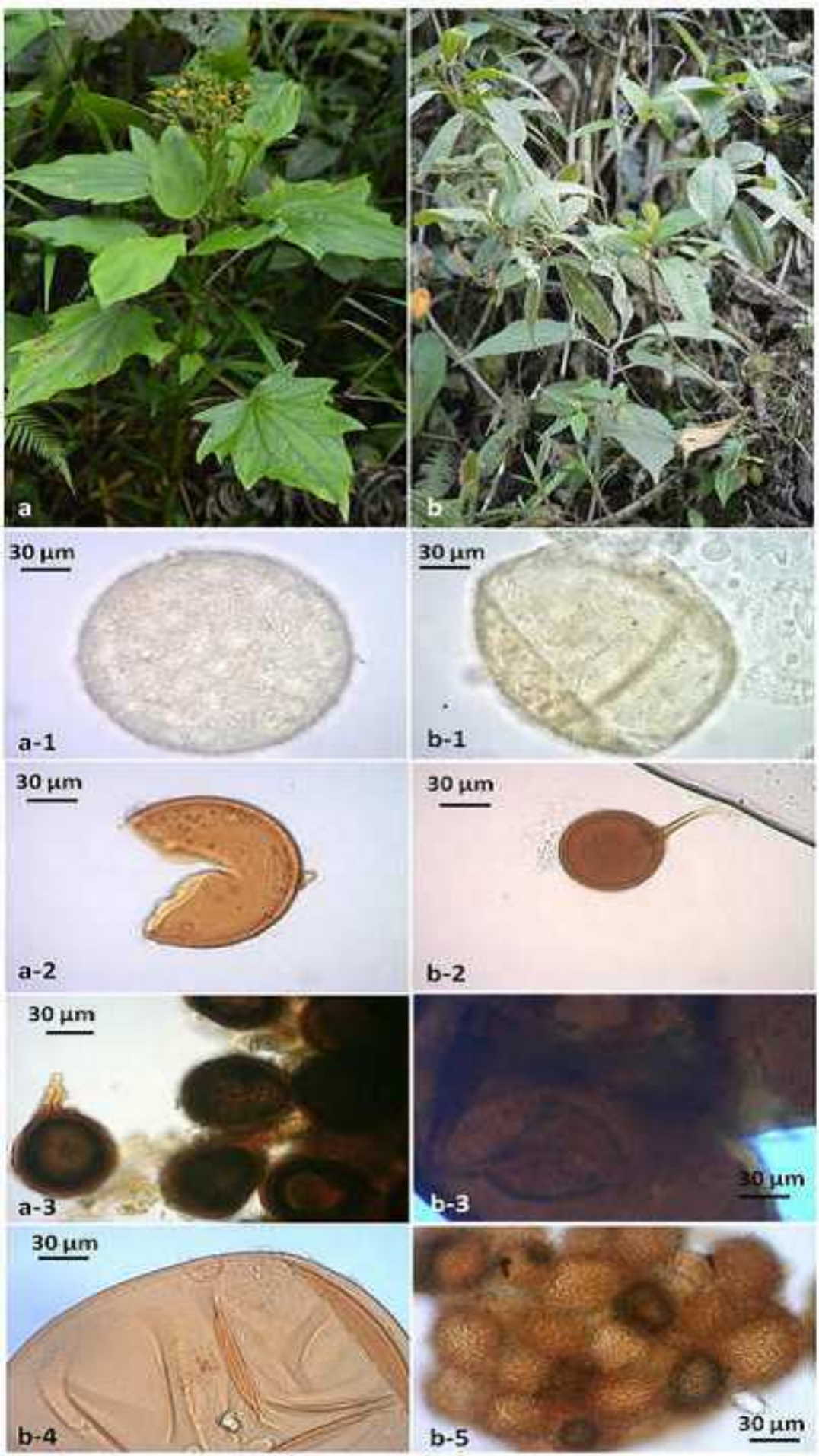

**Table**

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**Table 1.** Soil parameters from the sampling zones. NZ: natural mining zone; IZ: intensive mining zone. Values are expressed as mean of seven independent measurements ± standard deviation

| Soil parameter | Sampling zone | |
|---|---|---|
| | NZ | IZ |
| pH ($H_2O$) | 4.3 ± 0.5 | 4.1 ± 0.5 |
| SOM (%) | 2.8 ± 0.9 | 2.4 ± 0.5 |
| N (mg $kg^{-1}$) | 44.8 ± 6.5 | 85.3 ± 10.5 |
| P (mg $kg^{-1}$) | 6.9 ± 0.9 | 8.6 ± 2.3 |
| K (cmol $kg^{-1}$) | 0.14 ± 0.03 | 0.12 ± 0.07 |
| CEC (cmol $kg^{-1}$) | 6.3 ± 1.4 | 6.9 ± 1.2 |
| Fe (mg $kg^{-1}$) | 505 ± 154 | 664 ± 207 |
| Hg (mg $kg^{-1}$) | 0.19 ± 0.09 | 4.8± 1.2 |
| S (mg kg-1) | 33.2 ± 3.9 | 123.1 ± 41.5 |