Interreg Sudoe

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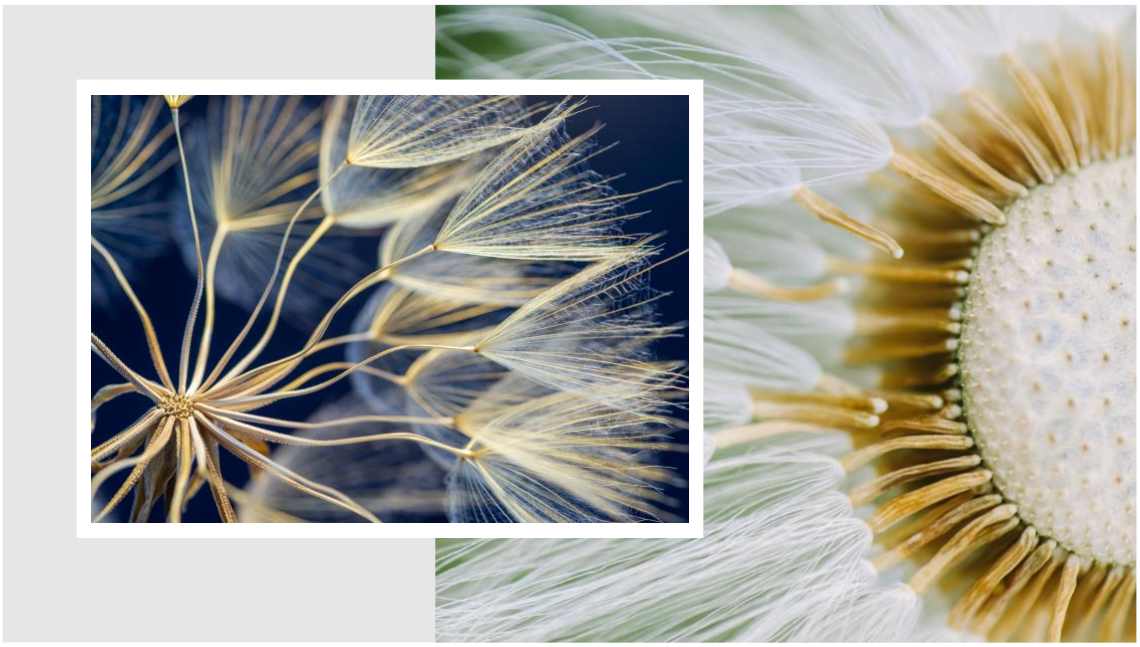
Phy2SUDOE

European Regional Development Fund

RECOVERING CONTAMINATED SOILS THROUGH PHYTOMANAGEMENT IN SOUTHWEST EUROPE



PRODUCT 3.2

COLLECTION OF GERMPLASM FROM METALLOPHYTIC AND METALLICOLOUS PLANTS

GT3 - Identification and Conservation of endemic biodiversity of contaminated sites for potential exploitation in biotechnological applications

This product has been produced under the Phy2SUDOE project (SOE4/P5/E1021):

"*Advancing in the application of innovative phytomanagement strategies in contaminated areas of the SUDOE space*"

Funded by the European Regional Development Fund (European Commission) through the 4th Interreg Sudoe Programme

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2023

CONTENTS

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

1. INTRODUCTION

Metal(loid)-contaminated sites are characterized by the presence of specific plant species that have adapted to thrive in such environments. Some of these species, known as metallophytes, have evolved specialized metabolic mechanisms to exclude, accumulate, or hyperaccumulate pollutants. Metallophytes are relatively scarce, as they are restricted to specific habitats and often endemic to these substrates and associated habitats, resulting in their classification as rare plants with limited geographic ranges. In contrast, certain species exhibit the ability to tolerate high concentrations of metal(loid)s through ecological plasticity, allowing them to inhabit diverse habitats and substrates. These plants are referred as metallicolous plants.

These exceptional plant species can endure and prosper in high-metal(loid) environments, demonstrating remarkable resilience and genetic adaptations. Safeguarding and conserving these species is crucial, not only for their intrinsic value but also for their potential contributions to phytoremediation and ecological restoration endeavors, as they contribute to the stabilization and reduction of available metal(loid)s in excess in the soil. Moreover, metallophytes and metallicolous plants serve as valuable resource for biodiversity conservation, as they support diverse ecological niches and provide habitats and food sources for other organisms in these challenging environments.

Therefore, to safeguard the distinctive biodiversity and conservation-worthy plant species in these regions, it is vital to protect the genetic material that embodies the unique genetic legacy of these specific species. In pursuit of this goal, a seed collection of metallophytes and metallicolous plants were undertaken, enabling their *ex situ* preservation at the Germplasm Bank of the Olarizu Botanical Garden in Vitoria-Gasteiz, Basque Country, Spain, under the direction of Agustí Agut Escrig.

2. IMPORTANCE OF METALLOPHYTIC AND METALLOPHILIC PLANTS PRESERVATION IN GERMPLASM BANKS

A germplasm bank, also known as a seed bank or gene bank, is a facility or repository that stores and preserves the genetic material (germplasm) of plants, including seeds, spores, or tissue cultures. Its primary purpose is to conserve the genetic diversity of different plant species for future use in research, breeding, conservation, and other applications.

Germplasm banks play a crucial role in safeguarding the genetic resources of plants, protecting them from potential threats such as habitat loss, climate change, pests, diseases, or human activities. The stored genetic material represents a living collection of diverse plant species, varieties, and populations. The importance of a germplasm bank of metallophytes and metallicolous plants lies in several key aspects, as follows:

- **Conservation of Genetic Diversity:** metallophytes and metallicolous plants often possess unique genetic traits and adaptations that allow them to tolerate and accumulate high levels of metal(loid)s. The genetic diversity of these species can be preserved in a germplasm bank, ensuring the long-term survival of their unique genetic traits and potential for future research and utilization.
- **Protection of Endemic and Rare Species:** many metallophytes are endemic to specific metal(loid)-contaminated habitats and are considered rare. A germplasm bank provides a means to safeguard these rare and endemic species, protecting them from potential threats such as habitat destruction, or climate change.
- **Research and Conservation Efforts:** a germplasm bank serves as a valuable resource for scientific research on metallophytes and metallicolous species. It allows researchers to study the genetic, physiological, and biochemical mechanisms underlying metal(loid) tolerance and accumulation. This knowledge can be applied to conservation efforts, ecosystem restoration, and the development of sustainable mining practices.
- **Ecological Restoration and phytoremediation:** metallophytes and metallicolous species and their microbiome play a crucial role in ecological restoration and phytoremediation of metal(loid)-contaminated sites. By

preserving their genetic material in a germplasm bank, these species can be utilized for the re-establishment of vegetation in degraded areas, enhancing biodiversity, and facilitating the remediation of metal(loid)-polluted soils.

- **Plant Breeding and Crop Improvement:** the genetic resources stored in a germplasm bank can be utilized for plant breeding programs and crop improvement efforts. The unique traits and adaptations of metallophytes may hold valuable genetic information that can be incorporated into breeding programs to develop new crop varieties with enhanced tolerance to metal stress or improved phytoremediation capabilities.
- **Education and Awareness:** a germplasm bank of metallophytes and metallicolous plants can also serve as an educational resource, raising awareness about the importance of biodiversity conservation, ecological resilience, and sustainable management of metal(loid)-contaminated environments. It can provide a platform for public outreach and engagement, promoting understanding and appreciation of the value and significance of these unique plant species.

3. COLLECTION OF SEEDS AND THEIR PRESERVATION IN THE GERMPLASM BANK OF THE OLÁRIZU BOTANICAL GARDEN

Seeds from the main metallophytes (endemic and of conservation interest) and other mesophilic habitat species that were considered relevant to be represented in the Germplasm Bank of the Olarizu Botanical Garden (OLA-BG) were collected from Lanestosa Mine (Basque Country, ES), Sentein-Bulard Mine (Ariège, FR), and Bandeira, Serra do Caréon (Galicia, ES) (Tables 1, 2 and 3).

The collection process followed the requirements and standards outlined in the primary methodologies for plant germplasm collection, such as those established by Bachetta et al. (2008), ENSCONET (2009), and Ferrer and Ferrando (2013). These methodologies ensure sample representativeness and appropriate sample sizes.

Table 1: Seeds collected in Lanestosa and preserved in Germplasm Bank of the Olarizu Botanical Garden

Accesión	Taxón	Familia	Fecha recolección	Localidad
032/2022	*Agrostis capillaris* L.	*Poaceae*	29/06/2022	Bocamina 2
046/2021	*Blackstonia perfoliata* (L.) Huds.	*Gentianaceae*	21/07/2021	Coto Txomin
006/2022	*Calluna vulgaris* (L.) Hull	*Ericaceae*	06/04/2022	Bocamina 2
026/2021	*Cardamine hirsuta* L.	*Brassicaceae*	05/03/2021	Coto Txomin
035/2021; 041/2022; 020/2022; 027/2022	*Cytisus cantabricus* (Willk.) Rchb.f. & Beck	*Fabaceae*	01/07/2021; 21/07/2021; 20/06/2022; 29/06/2022	Bocamina 2; Coto Txomin
028/2022	*Danthonia decumbens* (L.) DC	*Poaceae*	29/06/2022	Sismógrafo
007/2022	*Erica ciliaris* L.	*Ericaceae*	06/04/2022	Bocamina 2
008/2022	*Erica cinerea* L.	*Ericaceae*	06/04/2022	Bocamina 2
043/2021	*Euphrasia stricta* D. Wolff *subsp. stricta*	*Scrophulariaceae*	21/07/2021	Coto Txomin
152/2021; 026/2022	*Festuca rubra* L.	*Poaceae*	21/07/2021; 29/06/2022	Sismógrafo; Bocamina 2
044/2021	*Jasione montana* L.	*Campanulaceae*	21/07/2021	Bocamina 2
030/2022	*Leucanthemum gaudinii* subsp. *cantabricum* (Font Quer & Guinea) Vogt	*Asteraceae*	29/06/2022	Coto Txomin
014/2022	*Plantago lanceolata* L.	*Plantaginaceae*	26/05/2022	La Hoya
047/2021	*Polygonatum odoratum* (Mill.) Druce	*Asparagaceae*	21/07/2021	Sismógrafo
042/2021	*Pseudoarrenatherum longifolium* (Thore) Rouy	*Poaceae*	21/07/2021	Bocamina 2
027/2021; 034/2021	*Rumex acetosa* L.	*Polygonaceae*	11/06/2021; 01/07/2021	Sismógrafo
033/2022	*Saxifraga trifurcata* Schrad.	*Saxifragaceae*	29/06/2022	Bocamina 2
029/2021; 016/2022	*Scilla verna* Huds.	*Caryophylaceae*	11/06/2021; 26/05/2022	Sismógrafo, Bocamina 2
045/2021	*Scrophularia crithmifolia* Boiss.	*Scrophulariaceae*	21/07/2021	Coto Txomin
021/2022	*Silene nutans* L.	*Caryophylaceae*	20/06/2022	Coto Txomin
033/2021	*Silene vulgaris* (Moench) Garcke	*Caryophylaceae*	01/07/2021	Bocamina 2
031/2022	*Simethis mattiazzii* (Vand.) Sacc.	*Liliaceae*	29/06/2022	Bocamina 2
037/2021	*Sisymbrium austriacum* Jacq.	*Brassicaceae*	01/07/2021	Bocamina 2
029/2022	*Teucrium scorodonia* L.	*Lamiaceae*	29/06/2022	Coto Txomin
004/2021; 005/2021; 019/2021; 025/2021; 028/2021; 036/2021; 009/2022; 013/2022; 015/2022	*Thlaspi caerulescens* J.Presl & C.Presl	*Brassicaceae*	21/01/2020; 05/03/2021; 31/03/2021; 19/05/2021; 11/06/2021; 01/07/2021; 06/04/2022; 18/05/2022; 26/05/2022	Coto Txomin; Sismógrafo, La Hoya
005/2022	*Ulex gallii* Planch.	*Fabaceae*	06/04/2022	Sismógrafo

Table 2: Seeds collected in Sentein-Bulard and preserved in Germplasm Bank of the Olarizu Botanical Garden.

Accesión	Taxón	Familia	Fecha recolección	Localidad
226/2021	*Armeria muelleri* A.Huet	*Plumbaginaceae*	21/09/2021	Chichoué, Sentein
222/2021	*Armeria muelleri* A.Huet	*Plumbaginaceae*	20/09/2021	Le Bocard, Sentein
220/2021	*Campanula rotundifolia* L.	*Campanulaceae*	20/09/2021	Le Bocard, Sentein
221/2021	*Gypsophila repens* L.	*Caryophyllaceae*	20/09/2021	Terril, Sentein
218/2021	*Hutchinsia alpina* (L.) R.Br.	*Brassicaceae*	20/09/2021	Le Bocard, Sentein
223/2021	*Hutchinsia alpina* (L.) R.Br.	*Brassicaceae*	20/09/2021	La Plagne, Sentein
219/2021	*Hutchinsia alpina* (L.) R.Br.	*Brassicaceae*	21/09/2021	Chichoué, Sentein
224/2021	*Minuartia verna* (L.) Hiern	*Caryophyllaceae*	20/09/2021	Le Bocard, Sentein
225/2021	*Silene vulgaris* subsp. *prostrata* (Gaudin) Schinz & Thell.	*Caryophyllaceae*	21/09/2021	Chichoué, Sentein
227/2021	*Silene vulgaris* subsp. *prostrata* (Gaudin) Schinz & Thell.	*Caryophyllaceae*	21/09/2021	Chichoué, Sentein

Table 3: Seeds collected in Bandeira, Serra do Caréon and preserved in Germplasm Bank of the Olarizu Botanical Garden.

Accesión	Taxón	Familia	Fecha recolección	Localidad
003/2023	*Thlaspi caerulescens* J.Presl & C.Presl	*Brassicaceae*	06/06/2022	Campomarzo, Silleda, Pontevedra
004/2023	*Silene uniflora* Roth.	*Caryophyllaceae*	06/06/2022	Campomarzo, Silleda, Pontevedra
005/2023	*Silene uniflora* Roth.	*Caryophyllaceae*	07/07/2022	Campomarzo, Silleda, Pontevedra

The following figures showcase a selection of plants from which seeds were collected and subsequently preserved in the germplasm bank:

Figure 1: *Noccaea caerulescens* (hyperaccumulator; can grow in ultrabasic soils); Bandeira and Lanestosa; photo © Beatriz Rodríguez-Garrido, CSIC)

Figure 2: *Silene uniflora* (metal(loid) accumulator; Bandeira; photo © Beatriz Rodríguez-Garrido, CSIC).

Figure 3: *Cytisus cantabricus* (endemic species; metal(loid) excluder; Lanestosa site; photo ©CEA)

Figure 4: *Jasione montana* (metal(loid) accumulator; protected in Europe (92/43/ECC) and in Spain (RD 139/2011); Lanestosa; photo ©CEA)

Figure 5: *Armeria muelleri* (metal(loid) accumulator; Seinten; photo © Mench INRAE /Delerue Bordeaux INP)

Figure 6: *Helictrotrichon cantabricum* (endemic species to Spain (N) and France (S); metal(loid) excluder; Lanestosa; photo ©CEA)

Figure 7: *Erica arborea* (metal(loid) excluder; Borralha; photo ©UCP)

Figure 8: *Minuartia verna* (metal(loid) excluder; Sentein; photo ©Mench INRAE/Delerue Bordeaux INP)

The Germplasm Bank at Olárizu Botanical Gardens is equipped with the necessary scientific-technical staff, facilities, and equipment for the processing and long-term preservation of seeds. Each seed was assigned a unique accession number, which represents the sequential arrival of seed or spore samples at the Germplasm Bank facilities. The accession number is associated with various collection data, including the collection location, date, and collector. Additionally, characterization data such as seed weight and quantity, and morphometric measurements are recorded. This comprehensive process ensures the proper processing, preservation, and characterization of seed samples at the Germplasm Bank, while also facilitating plant production for conservation purposes within the various dedicated facilities at Olárizu Botanical Gardens.

Figure 9: Collection, processing, and preservation of seeds in Germplasm Bank of the Olarizu Botanical Garden (Vitoria-Gasteiz, Basque Country).

The Germplasm Bank Database of the Olárizu Botanical Garden stores all the information about the conserved accessions since its establishment in 2011. Similarly, herbarium samples obtained from the collected seeds bear the same collection data and are assigned a herbarium code after processing the sample.

4. DISSEMINATION OF THE GERMPLASM COLLECTION

The germplasm collection will be disseminated through various methods to ensure its availability and utilization by researchers, breeders, and conservationists, as follows:

A. **Seed Distribution:** the germplasm bank has mechanisms in place to distribute seeds or plant material to interested individuals or organizations. It has a formal seed distribution program where researchers or breeders can request specific accessions or species for their work. Seeds are typically packaged and sent to the recipients along with relevant documentation and information about the genetic material.

B. **Scientific Conferences and Workshops:** international conferences, workshops, and scientific meetings focused on plant genetic resources and biodiversity conservation provides opportunities for researchers, breeders, and conservationists to share and exchange germplasm material. These events often include germplasm exchange sessions or exhibitions where participants can showcase and exchange genetic material.

C. **Collaborative Networks and Consortia:** the germplasm collections can be disseminated through collaborations and partnerships between different institutions, organizations, or countries. These partnerships may involve sharing accessions or establishing reciprocal agreements to exchange genetic material. Collaborative networks allow for the broader dissemination of germplasm and facilitate the sharing of resources and knowledge among different entities.

D. **Online Platforms and Databases:** The Germplasm Bank of the Olarizu Botanical Garden (https://www.vitoria-gasteiz.org/) and Phy2SUDOE (https://www.phytosudoe.eu/en/) websites will describe the collection's details, including its objectives, available seeds, and associated data. The collection will also be registered in relevant online databases to expand its visibility to a wider research community. All images obtained for each species for morphometric measurements are available in the Xavier de Arizaga Digital Herbarium (herbario.ian-ani.org), which is free to access.

E. **Social Media and Online Presence:** Twitter, LinkedIn, or ResearchGate will be used to share updates, publications, and relevant information about the collection.

5. REFERENCES

- Abarca, J.M., Martínez, J.M. 2009. El catálogo florístico: una herramienta básica en la gestión. 5º Congreso Forestal Español. Sociedad Española de Ciencias Forestales.
- Aizpuru I., Aseginolaza, C., Uribe-Echebarria, P.M., Urrutia, P., Zorrakin, I. 1999. Claves ilustradas de la flora del País Vasco y territorios limítrofes. Servicio Central de Publicaciones del Gobierno Vasco/Eusko Jaurlaritza. Vitoria-Gasteiz.
- Alcaraz, F. 1996. Fitosociología integrada, paisaje y biogeografía. In: Loidi (ed.) Avances en Fitosociología: 59-94. Ed. UPV.
- ALCARAZ, F. 2013. El método fitosociológico. Geobotánica, Tema 11. Universidad de Murcia.
- Bachetta, G., Bueno, A., Fenu, G., Jimenez, B., Mattana, E., Piotto, B., Virevaire, M. 2008. Conservación *ex situ* de plantas silvestres. La caixa, Principado de Asturias.
- Bachetta, G., Bueno, A., Fenu, G., Jimenez, B., Mattana, E., Piotto, B., Virevaire, M. 2008. Conservación *ex situ* de plantas silvestres. La Caixa, Principado de Asturias.
- Baker, A., Ernst, W., Van der Ent, A., Malaisse, F., Ginocchio, R. 2010. Metallophytes: the unique biological resource, its ecology and conservational status in Europe, central Africa and Latin America. Ecology of Industrial Pollution. British Ecological Society.
- Barrera, I. 2017. La vegetación en los mapas. Memorias Real Sociedad Española de Historia Natural, 2ª ep., 14: 317-342.
- Barrutia, O., Artetxe, U., Hernández, A., Olano, J.M., García-Plazaola, J. I., Garbisu, C., Becerril, J.M. 2011. Native plant communities in an abandoned Pb-Zn mining area of northern Spain: Implications for phytoremediation and germplasm preservation. International Journal of Phytoremediation, 13:256–270, 2011.
- Becerril, J.M. 2019. Colaboración entre el Centro de Estudios Ambientales (CEA) y el grupo de investigación EKOFISKO del Departamento de Biología Vegetal y Ecología de la Universidad del País Vasco (UPV/EHU) para el desarrollo de actividades de investigación en material de fitorremediación aplicada en el término municipal de Vitoria-Gasteiz. Disponible en: https://blogs.vitoria-gasteiz.org/ceagreenlab/files/2020/09/2-INFORME-FINAL-UPV-EHU-GDL_compressed.pdf
- Biurrun, I., García-Mijangos, I., Loidi, J., Campos, J.A. & Herrera, M. 2011. La vegetación de la Comunidad Autónoma del País Vasco. Leyenda del mapa de series de vegetación a escala 1: 50.000. Eusko Jaurlaritza – Gobierno Vasco, Lejona (Vizcaya). 197 pp.
- Bizoux, J. P., Brevers, F., Meerts, P., Graitson, E., Mahy, G. 2004. Ecology and conservation of Belgian populations of *Viola calaminaria*, a metallophyte with a restricted geographical distribution. Belgian Journal of Botany 137, 91–104.

- Braun-Blanquet, J. 1979. Fitosociología. Bases para el estudio de las comunidades vegetales. Blume. Madrid. 820 pp.
- Castroviejo, S. (coord. gen.). 1986-2012. Flora iberica 1-8, 10-15, 17-18, 21. Real Jardín Botánico, CSIC, Madrid.
- Ensconet. 2009. Ensconet seed collecting manual for wild species.
- Ensconet. 2009. Ensconet Seed Collecting Manual for Wild Species.
- Ferrer, PP., Ferrando, I., Gago, C., y Laguna, E. 2013. Manual para la conservación de germoplasma y el cultivo de la flora valenciana amenazada. Colección manuales técnicos biodiversidad, 3. Conselleria d´infraestructures, territori i medi ambient. Generalitat valenciana. Valencia.
- FERRER, PP., FERRANDO, I., GAGO, C., Y LAGUNA, E. 2013. Manual para la conservación de germoplasma y el cultivo de la flora valenciana amenazada. Colección Manuales Técnicos Biodiversidad, 3. Conselleria d´Infraestructures, Territori i Medi Ambient. Generalitat Valenciana. Valencia.
- Garbisu, C., Alkorta, I., Kidd, P., Epelde, l., & Mench, M. (2020). Keep and promote biodiversity at polluted sites under phytomanagement. Environmental science and pollution research, 27, 44820-44834.
- HERRERA, M. 1995. Estudio de la vegetación y flora vascular de la cuenca del río Asón (Cantabria). Guineana 1: 1-435. Universidad del País Vasco/Euskal Herriko Unibertsitatea.
- Hobbs, R.J., NORTON, D.A. 1996. Towards a conceptual framework for restoration ecology. Rest. Ecol. 4: 93–110.
- LOIDI, J., BIURRUN, I., HERRERA, M. 1997b. La vegetación del centro-septentrional de España. en Rivas-Martínez, S., Díaz, T.E., Penas, A., Fernández, F. (eds.) Itinera Geobotanica 9: 5-160. Asociación española de fitosociología. Universidad de León, León.
- LOIDI, J., DÍAZ GONZÁLEZ T.E., HERRERA, M. 1997a. El paisaje vegetal del centro-norte de España. Guía de la excursión. en Rivas-Martínez, S., Díaz, T.E., Penas, A., Fernández, F. (eds.) Itinera Geobotanica 9: 5-160. Asociación española de fitosociología. Universidad de León, León.
- Rivas-Martínez, S., Fernández-González, F., Loidi, J., Lousã, M., Penas, A. 2001. Syntaxonomical checklist of vascular plants communities of Spain and Portugal to association level. Itinera Geobotanica 14: 5-341.

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The PhytoSUDOE project (SOE4/P5/E1021) has been funded by the European Regional Development Fund (European Commission) through the 4th Interreg Sudoe Programme.

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).