

Co-funding of regional, national and international programmes (COFUND)

DOC2AMU THESIS PROJECT 2018 CALL FOR APPLICATIONS**Critical raw ELEMent Bio-Extraction (CELEBEX)****1. GENERAL INFORMATION**

Call	2018-25
Topic	Climate change
Keywords	Critical Raw Element, Waste Recycling, Bio-extraction, Phyto-extraction, Microbial Fuel cell, Metal speciation

2. THESIS DIRECTOR(S), RESEARCH UNITS AND DOCTORAL SCHOOLS

Thesis director	Mélanie AUFFAN
Research Unit	Centre Européen de Recherche et d'Enseignement des Géosciences de l'Environnement
Doctoral school	ED 251 - Sciences de l'Environnement
Thesis co-director	Wafa ACHOUAK
Research Unit	Laboratoire d'écologie microbienne de la rhizosphère et d'environnements extrêmes
Doctoral school	ED 062 - Sciences de la Vie et de la Santé

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DOC2AMU PROJECT 2018 CALL FOR APPLICATIONS

CELEBEX Critical raw ELEMENT Bio-EXtraction

1. DESCRIPTION OF THE PHD THESIS PROJECT

1.1 OBJECTIVES OF THE PROJECT BASED ON THE CURRENT STATE OF THE ART

Critical raw elements (CRE), including rare earths elements (REE) and platinoids, are at the heart of many emerging technologies. As an example, photovoltaic primarily based on Si technology is now evolving to Cd, Ga, Ge and Te. Wind turbines require the use of super-strength magnets made of Nd and other REEs. The strong demand for these elements for the development of "high-tech" products has led to a boom of raw materials never encountered before (Figure 1). At present, the main problem regarding those CRE is their supply due to their low concentrations in ores. Moreover, supply of these elements faces complex geostrategic conflicts. Their production is currently concentrated in China, which is also the main consumer, influencing their availability and price on the world market.

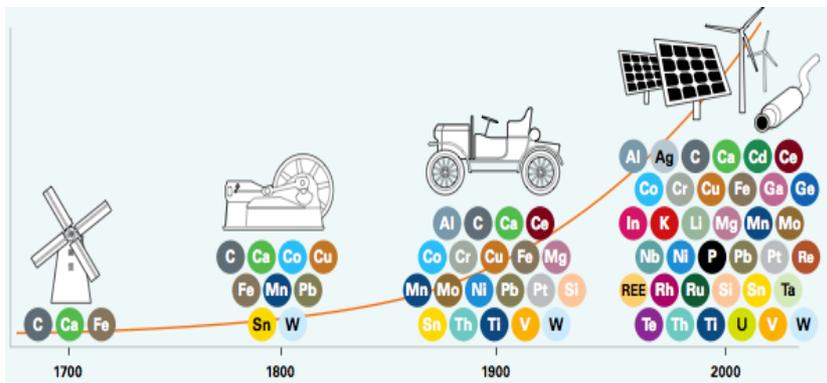


Figure 1: Increase of metal assemblage complexity with the development of renewable energies (Achzet et al. 2011).

The recent increase of CRE demand will lead to major environmental, industrial and geo-political issues. First, it will inevitably lead to their **environmental release**. It is difficult to predict how these elements could impact Environment and Human health since they were poorly investigated. **Industrial and geostrategic issues** also exist. Nd, used in 'super-strength' permanent magnet for most electric engines, is emblematic since it is produced at 90% by Chinese companies. Therefore the availability of elements essentials in high-tech applications is of the highest concern. Because of criticality of most of the CRE **recycling and recovery technologies** will play a central role. Although recycling routes for some specific wastes have already emerged (e.g. electrical and electronic equipment wastes), other ultimate wastes produced in large quantities are potentially good candidates for the extraction of these strategic elements (e.g. fly ash from municipal solid waste incineration). Typical recycling processes used at an industrial scale usually involved high temperature treatment and/or the use of important quantities of acidic or basic solvents. In the optic of reducing the environmental fingerprint of the recycling process **this project aims at developing bio-extraction processes** alternative to more traditional pyro- and hydrometallurgy.

Microbes play key active roles in the geosphere, particularly in element biotransformation and biogeochemical cycling, metal and mineral transformation, decomposition, bioweathering, and soil and sediment formation (Gadd, 2010). Thus microbes play important roles in the mobilization or immobilization of metals and can therefore be of great importance in heavy metals availability to plants by modulating metal speciation, enhancing metal uptake or alleviating metal stress (Miransari, 2011). In soil and in the rhizosphere, microbial populations can modulate metal mobility and availability to the plant through the release of chelating agents (*e.g.* siderophores, exopolymers such as exopolysaccharides), redox changes, phosphate solubilization (Jing et al. 2007).

The unique ability of bacteria to interact with metals has been operated to extract industrial metals from primary ores in leaching or bio-oxidation processes (Johnson, 2014; Gadd, 2000), currently referred as biometallurgy (Zhuang et al., 2015). As in the geosphere and rhizosphere, the same mechanisms allow metal recovery by bacteria and are based on metal extraction by solubilization (*e.g.* acidolysis, redoxolysis, complexolysis) or by interaction with the gangue matrix to help unlock the metal (*e.g.* bio-oxidation). Biomining and bio-oxidation that are already practiced as industrial processes to extract metals from ores (Cu, Ni, Au..) are likely to contribute to the recovery of REEs (Zhuang et al., 2015).

Some improvements in CRE extraction rely on the release of metals of interest from the matrix, increase of CRE selectivity in the extraction process, but also compartmentalization of CREs for an easy recovery. For the bio-extraction process, we will generate a library of bacterial strains isolated from CRE-rich environments. **We will address two main pathways for CRE recovery based on a process, using microbial fuel cells, and an environmental passive process, using phyto-extraction.**

Microbial fuel cells (MFCs) are emerging sustainable technologies for waste to energy conversion. Instead of requiring metal catalysts, MFCs utilize bacteria that oxidize organic matter and either transfer electrons to the anode or take electrons from the cathode. These devices are thus based on a wide microbial diversity that can convert a large array of organic matter components into sustainable and renewable energy. A wide variety of explored environments were found to host electrogenic bacteria, including extreme environments. Using phosphogypsum sediments, as a source of bacteria and metals in a MFC device, we showed that specific bacteria were able to carry cathodic reactions by using solid electron donors to reduce final electron acceptors such as oxygen and allowed recovering of many metals on the biocathode and monitoring interesting current density, in the absence of any carbon source addition. We propose to develop MFCs to recover rare earths metals and platinoids from CREs enriched wastes. The recovery of CREs from wastes by this technology has not been previously studied.

Phyto-based processes have been shown to efficiently take up certain metals (*e.g.* Tang et al, 2012; Souza et al, 2013; Sheoran et al, 2009; Ali et al, 2013). However, a better understanding of the fundamental mechanisms of CRE transfer from soil to plants is an essential pre-requisite for more efficient process development. Plants exert strong selection on the rhizosphere microbiota, which play important roles in plant growth (Haichar et al., 2008 ; Mendes et al., 2013) and metal uptake (Muehe et al, 2015 ; Abou-Shanab et al, 2008). Thus, understanding how root-associated microbiomes help managing CRE uptake by plant is crucial to determine if the manipulation of the rhizosphere could increase phytoextraction. The originality of this approach lies on the preliminary characterization of a natural field site (Jas Roux, Ecrin National Park) with high CREs geochemical background (“hot spots” of Ga, Sb, Nd, Tl, Ag, W, from 10 to 500 ppm) colonized by a variety of plants accumulating CREs in their leaves and excreting CREs in guttation fluids. The actual ecosystem functioning is the result of long-term processes that reached steady state.

1.2 METHODOLOGY

The proposed approach will be divided into 3 main tasks:

- **Task 1** will be devoted to the fine characterization of the wastes and the speciation of strategic elements to better optimize the bio-extraction processes. In this regard, multi-scale analysis will be performed on the wastes to identify the bearing phase and the atomic environment of the targeted elements (e.g. XRD, uXRD, uXRF, XANES, EXAFS). Two wastes will be selected based on preliminary results that showed a good potential (in term of total concentrations) for the recovery of some CREs (REEs, Antimony, Yttrium, etc). The first one is red mud residues that are by-products of the extraction of alumina, produced in Gardanne (France). The red mud residue will be provided by our partner Alteo. The second one will be an industrial or municipal waste provided by our partner Veolia or fly ashes from municipal solid waste from two incinerators located in the Marseille area (Toulon and Fos-sur-Mer, France). Preliminary experiments have been performed on fly ashes from these 2 incinerators and have shown some interesting concentration in antimony.

Deliverable 1. A report or a scientific paper on the characterization of the 2 wastes and speciation of selected CREs will be provided in which the best extraction strategy will be discussed.

- **Task 2** will explore the extraction of CREs from the two selected wastes based on microbial fuel cells built on the library of bacterial strains able to extract CREs. Sediments or soil from CREs enriched wastes as well as pure cultures will be used as source of inoculum in bioreactors. The MFCs will be electrochemically characterized through amperometry, cyclic voltammetry, voltage vs. current density (V–J) curves and power vs. current density (P–J) curves. Characterization of biofilm architecture will be achieved by different microscopic technics, and CREs could be localised on the surface of the electrode by laser ablation ICP-MS. The microbial composition of electroactive biofilm will be performed by PCR-based MiSeq technique to phylogenetically characterize the bacterial communities. We will also investigate the isolation of new electrogenic bacteria from efficient biofilms to decipher their electron transfer mechanisms and to determine their contribution to CREs recovery.

Deliverable 2. A report or a scientific paper on the extraction efficiency of CREs using microbial fuel cells will be provided.

- **Task 3** will be dedicated to the understanding of bio-extraction processes used by terrestrial plants together with their associated bacteria in a natural soil. Terrestrial plant and soils will be sampled in a natural hot spot (Jas Roux site, Parc des Ecrins, France) and an anthropic hot spot (Mangegari site, red muds storage site). Among the diversity of plants developing in CRE hot spots in Jas Roux, preliminary chemical results suggest a correlation between CREs (Sb, Tl) concentrations in soils and in leaves of *Festuca laevigata* and *Saxifraga paniculata*. Based on the ability to extract CREs or unlock CREs from the matrix, a set of bacterial strains will be selected. These bacteria will be re-inoculated to the soil to increase CRE phytoavailability. Non-inoculated soils will be used as controls and as reportings of the steady state in Jas Roux. Plants inoculated or non-inoculated will be grown in the Jas Roux soil. The concentration of CREs accumulated in leaves will be determined by ICP-MS. XAS will help deciphering HTM speciation in leaves.

Deliverable 3. A report or a scientific paper on the extraction efficiency of CREs by plants grown on natural and anthropic hot spots will be provided.

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1.3 WORK PLAN

	0 month	6 months	12 months	18 months	24 months	36 months
Task 1						
Task 2						
Task 3						
Writing						

Figure 2: Chronogram of the activities.

1.4 SUPERVISORS AND RESEARCH GROUPS DESCRIPTION

UMR 7330 CEREGE - Interfaces and transfer (InterfasT) team is known as an expert group in **molecular environmental science** and especially regarding the **reactivity of metals in different environmental compartment** (soil, water, biomass) **and wastes**. M. Auffan, C. Levard and J. Rose have a strong experience in the characterization of metals using various X-ray based techniques such as X-ray Absorption Spectroscopy at various synchrotron beamlines : ESRF (Grenoble, France), SOLEIL (Paris, France), SLS (Villigen, Switzerland), SSRL (San Francisco, US), APS (Chicago, US) and ELLETRA (Trieste, Italy)

UMR 7265 LEMIRE - Research in LEMiRE aims at identifying the components of molecular dialog between plant and bacteria in the rhizosphere. The team is comprised with two Bio-IT engineers expert in metagenomic data treatment, microbial ecologists, and chemists. UMR7265 is equipped with sophisticated plant growth chambers (IBIZA labeled). Our group has a wide experience in the study of plant-microbe interaction in the rhizosphere and in molecular characterization of microbial community structure. Partners W. Achouak (Microbiologist of the rhizosphere) and C. Santaella (expert in metal tolerance)

2. 3I DIMENSIONS AND OTHER ASPECTS OF THE PROJECT

2.1 INTERDISCIPLINARY DIMENSION

The strength of the project relies on the interdisciplinary (mineralogy, physico-chemistry, microbiology) and multi-scale approach based as well on field campaign than on up-to-date characterisation platforms and tools. The difficulties to manage such an interdisciplinary PhD project were solved during previous PhD (e.g. Astrid Avelan, Mohamed Barakat) and several joint publications (see the recent publication section) that attest our efficient collaboration.

CEREGE has a unique expertise in the characterisation of metal speciation in various matrices, their biotransformation and biodistribution. They are affiliated to the Graduate School 'Science de l'Environnement ED251'. Wafa Achouak is an expert in microbiology and gene regulation and expression. She is affiliated to the Graduate School 'Science de la vie et de la santé ED62'.

2.2 INTERSECTORAL DIMENSION:

This proposal aims at opening new scientific perspectives regarding upcoming CRE supply. Indeed, the fast -growing demand of CRE in relation with renewable energy and high tech technology will bring new societal and scientific challenges. Improving a reliable access to strategic elements is a prominent challenge for resource-dependent countries like France and results from this PhD would provide valuable information to help closing the Critical raw material cycles.

This study will answer three objectives of the Synthesis of the Research and Innovation Strategies for Smart Specialisation (SRI-S3) of Provence-Alpes-Côtes d'Azur. We will participate to (i) the promotion of global environmental monitoring and crisis management solutions, (ii) the development of the production of renewable energy, (iii) the preparation of strategic areas of activities of the future.

The presence of Alteo and Veolia as partners of the project will insure an optimized connection with the societal demand. They will provide red mud residues and municipal or industrial wastes that present an important economic potential for recycling. With their help, we will address two of the weaknesses in Provence-Alpes-Côtes d'Azur identified by the SRI-S3, the insufficiently exploited scientific excellence, and the lack of innovation tools to support the market.

Finally, dissemination and valorization of the main results will be performed in collaboration with our partner Éa écoentreprises. This régional cluster based on cleantech aims to create synergies between local authority, scientists and socio-economic actors (SMEs) which is the keystone of a successful transition from a linear to circular economy. Éa represents 140 members, (éco-technology and éco-services) mainly localized in the Provence Alpes Côte d'Azur region, and especially in the Aix-Marseille Métropole, including SMEs (EODD, ERG, BURGAP, agrosylva, Blueset, Syntea., Esterel..) and large companies (Pizzorno, SEM, SERAMM, Antea group etc.). Éa has long experience to promote innovation and to create synergies in the ecosystem clean tech. Working groups will be organized to discuss the outputs of the project and to create new synergies between academic and socio-economic partners to favor multi-sectorial collaboration and to respond to new calls for projects related to circular economy (e.g. European funding).

2.2 INTERNATIONAL DIMENSION:

This project will be performed in collaboration with Helen Hsu-Kim (associate professor at Duke University, North Carolina, US) who is interested in the recovery of critical metals from fly ash from coal combustion. Visiting students allows the shearing of protocols and strategies to optimize the characterization and extraction of metals from wastes. We plan to pursue these exchanges in the context of this project.

We are on the process of joining the COST program led by Antonio Cobello on "Network on technology-critical elements - from environmental processes to human health threats" and the COST program led by Kerstin Kuchta on "European network for innovative recovery strategies of rare earth and other critical metals from electric and electronic waste" which will facilitate new collaborations with European partners.

Finally, results will be presented in international conferences and the results will be published in international peer-reviewed journals.

3. RECENT PUBLICATIONS

- Auffan M**, Tella M, **Santaella C**, Brousset L, Pailles C, Barakat M, Espinasse B, Artells E, Issartel J, Masion A, **Rose J**, Wiesner M, **Achouak W**, Thiery A, Bottero J-Y (2014) An Adaptable Mesocosm Platform for Performing Integrated Assessments of Nanomaterial Risk in Complex Environmental Systems. *Scientific reports* 4, 5608.
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4. EXPECTED PROFILE OF THE CANDIDATE

Candidates should have a master degree with a background in biology and/or chemistry. Prior experience/knowledge in both fields is preferred but not required. Specific knowledge should include one or more of the following aspects: inorganic chemistry, electrochemistry, microbial ecology or in microbiology, the use of experimental tools dedicated to the characterization of critical raw elements, solid matrices (wastes, soils). A previous research experience in the lab working on one of the aspect cited above is recommended. Good communication skills (oral and writing) and a good English and/or French level are required. Recommendation letters are strongly recommended.

5. SUPERVISORS' PROFILES

Director: Melanie Auffan is a CNRS senior scientist at the CEREGE (European Geosciences Center) in Aix en Provence (France). She is member of the steering committee of CEINT Centre for the Environmental Implications of Nanotechnology steering committee and she is part of the Labex SERENADE Safer Ecodesign Research and Education applied to NANomaterial Development. Her research addresses the physico-chemical properties and surface reactivity of metal-based nanominerals in contact with living organisms. She obtained her HDR in 2016 (Bio-physical-chemical mechanisms of interactions between nanomaterials and living organisms: an interdisciplinary and multi-scale approach). Graduate students supervised: Amalia Turner (2014-2017), Clément Layet (2014-2017, Layet et al. 2017), Lauren Barton (2010-2014, publications: Barton et al. 2014a, 2014b, 2015a, 2015b).

Co-director 1: Wafa Achouak is Research Director at CNRS and has led the Laboratory of Microbial Ecology of the Rhizosphere and Extreme Environment (LEMIRE) at CEA Cadarache since 2004. Her present research focuses on adaptive response, regulation and expression of phytobeneficial traits of plant root bacteria, and their exploitation for environmentally friendly agriculture applications. She has been involved in the development of microbial fuel cells since 2005. She has supervised 18 PhD.

Co-director 2: Clément Levard is a CNRS research scientist at the CEREGE (European Geosciences Center) in Aix en Provence (France). Physical chemist, he is interested in the physico-chemical properties of metals and nano-structured materials in an environmental context. To develop these aspects, he specialized in synchrotron-based techniques using X-rays including total diffusion, standing waves and absorption spectroscopy. He is a member of synchrotron SOLEIL Peer Review Committee « Ancient Materials, Environment and Earth ». He is also an expert for OECD regarding the stability of nano-structured materials in environmental waters. He has published 40 peer-reviewed articles in international journals in the field. Graduate students supervised: Maureen Le Bars (2015-2018), Cyprien Mauroy (2014-2017), Astrid Avellan (2012-2015, 5 publications: Avellan et al. 2014, 2016a, 2016b, 2016c, 2017)

AVIS DES DIRECTEURS DES LABORATOIRES CONCERNES PAR LE PROJET DE THESE

**Avis du directeur du laboratoire du
directeur de thèse, M. NOM Prénom**

THOUVENY Nicolas
 Favorable Défavorable

Commentaires :

Fait à Marseille, le 21.12.17

Signature

NICOLAS THOUVENY

Directeur du CEREGE

CEREGE

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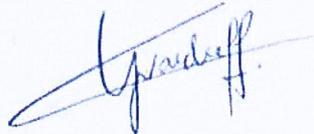
**Avis du directeur du laboratoire
du co-directeur de thèse,
M. CHAGVARDIEFF Pierre**

Favorable Défavorable

Commentaires :

Fait à Marseille, le 22/12/2017

Signature





Soutien au projet - CREBEX
(Critical Raw Element Bio-extraction)

Maisons-Laffitte, le 3 janvier 2018

Monsieur,

Par la présente, sans être partenaire du projet CREBEX, nous souhaitons confirmer notre intérêt pour la candidature du CEREGE (UMR 7330) associé au LEMIRE (UMR 7265) pour le projet de thèse sur l'extraction des métaux stratégiques critiques par des technologies de bio-extraction sur des déchets.

Cette démarche s'inscrit pleinement dans notre stratégie industrielle. Acteur de l'économie circulaire, Veolia met au point des solutions innovantes pour accroître le taux de recyclage et de valorisation des déchets, sous forme de matière ou d'énergie. Nous développons des filières de valorisation qui permettent de réintroduire ces déchets ou des matières premières secondaires dans de nouveaux cycles de consommation ou de production devenant ainsi de nouvelles ressources.

Ce projet de thèse s'inscrit pleinement dans notre démarche d'innovation, notre ancrage territorial historique et la volonté de coopération que nous souhaitons développer entre le CEREGE et Veolia Recherche et Innovation (VERI).

En effet, nous avons vu dans ce projet partenarial l'occasion de favoriser de futurs échanges sur des solutions innovantes en lien avec nos métiers, notamment dans la valorisation matière des déchets issus des collectes, des filières de traitement des déchets solides et liquides d'origine municipale et/ou industrielle.

Potentiellement, nous pourrions accompagner certains volets de cette thèse qui serait portée par le CEREGE, en créant des synergies interdisciplinaires utiles à l'émergence de solutions innovantes, économiquement viables.



François ENGUEHARD
Directeur des Centres de Recherche

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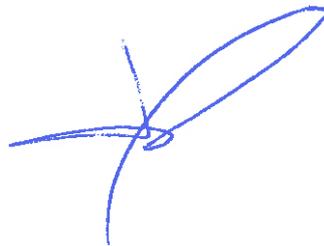
Objet : projet de Thèse DOC2AMU

Madame, Monsieur,

Le projet de thèse en question, coordonné par mesdames Mélanie Auffan, Wafa Achouack et monsieur Clément Levard vise à extraire des métaux critiques dans différents types de déchets en utilisant des protocoles d'extraction qui présentent un impact environnemental réduit par rapport aux modes d'extraction couramment utilisés. Les résidus d'extraction d'alumine produit par Alteo sont potentiellement de bons candidats pour l'extraction de ces métaux. Lors d'un stage de master 2 encadré par monsieur Clément Levard, les teneurs en métaux critiques dans ces résidus ont pu être mesurées et ont montré, pour certains métaux, des concentrations intéressantes pour envisager leur extraction. Le procédé Bayer utilisé pour l'extraction de l'aluminium a pour effet d'enrichir d'un facteur 2 à 3 certains métaux dans ces résidus. Au regard de ces premiers résultats, nous soutenons le projet de thèse DOC2AMU qui vise à valoriser ces résidus en étudiant leur potentiel pour la récupération de métaux critiques. Nous nous engageons à fournir des échantillons de résidus dans le cadre de ce travail de thèse.

Laurent Poizat

Chef de Projets





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M. Clément LEVARD
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InterFast
Labex SERENADE

Aix-en-Provence, le 21 décembre 2017

Objet : Lettre de soutien au projet de thèse axée sur la bio extraction de métaux critiques

Monsieur,

A la lecture du projet de thèse que votre organisme de recherche, associé au LEMIRE, nous soumet et au regard des enjeux économiques et écologiques auxquels ce sujet de recherche répond, je vous confirme le soutien de notre réseau d'éco-entreprises.

Éa éco-entreprises œuvre en effet depuis plusieurs années au transfert de technologies innovantes, initiées par les centres de recherche et visant une amélioration de la compétitivité des éco-entreprises.

Les métaux critiques revêtent un caractère stratégique pour l'économie : ils sont aujourd'hui présents dans beaucoup de produits de consommation courante et dans de nombreuses technologies à haute valeur ajoutée, parmi lesquels on retrouve plusieurs filières des éco-activités, dont le photovoltaïque. A l'heure actuelle, la concentration géographique des gisements en production crée un risque d'approvisionnement, certains pays producteurs en positions monopolistique ayant adopté des orientations stratégiques qui limitent leurs échanges commerciaux : c'est notamment le cas de la Chine.

Face à ce risque, il est nécessaire aujourd'hui de trouver de nouveaux gisements et de travailler sur la valorisation de ces métaux critiques.

Le réseau compte actuellement plus d'une dizaine d'entreprises utilisatrices de métaux ou en capacité de mettre en œuvre de nouvelles techniques de valorisation qui peuvent être bénéficiaires des résultats de la thèse proposée et notre cluster peut travailler à la dissémination de ces résultats au-delà de ces entreprises.

Nous suivrons donc avec grand intérêt le suivi de ce projet dont vous voudrez bien nous faire part. Vous souhaitant bonne réception de la présente, nous vous prions de croire, Monsieur, en l'assurance de nos sincères salutations.

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