EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Brussels, 24 March 2020

COST 019/20

DECISION

Subject: **Memorandum of Understanding for the implementation of the COST Action “Trace metal metabolism in plants” (PLANTMETALS) CA19116**

The COST Member Countries and/or the COST Cooperating State will find attached the Memorandum of Understanding for the COST Action Trace metal metabolism in plants approved by the Committee of Senior Officials through written procedure on 24 March 2020.

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MEMORANDUM OF UNDERSTANDING

For the implementation of a COST Action designated as

COST Action CA19116
TRACE METAL METABOLISM IN PLANTS (PLANTMETALS)

The COST Member Countries and/or the COST Cooperating State, accepting the present Memorandum of Understanding (MoU) wish to undertake joint activities of mutual interest and declare their common intention to participate in the COST Action (the Action), referred to above and described in the Technical Annex of this MoU.

The Action will be carried out in accordance with the set of COST Implementation Rules approved by the Committee of Senior Officials (CSO), or any new document amending or replacing them:

a. “Rules for Participation in and Implementation of COST Activities” (COST 132/14 REV2);
b. “COST Action Proposal Submission, Evaluation, Selection and Approval” (COST 133/14 REV);
c. “COST Action Management, Monitoring and Final Assessment” (COST 134/14 REV2);
d. “COST International Cooperation and Specific Organisations Participation” (COST 135/14 REV).

The main aim and objective of the Action is to tackle basic and applied issues related to trace metal deficiency or excess levels in plant physiology and crop production by the combined expertise of physiologists, (bio)physicists, (bio)(geo)chemists, molecular geneticists, ecologists, agronomists and soil scientists. Knowledge will be translated to the needs of farmers and consumers, with inputs from companies. This will be achieved through the specific objectives detailed in the Technical Annex.

The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 80 million in 2019.

The MoU will enter into force once at least seven (7) COST Member Countries and/or COST Cooperating State have accepted it, and the corresponding Management Committee Members have been appointed, as described in the CSO Decision COST 134/14 REV2.

The COST Action will start from the date of the first Management Committee meeting and shall be implemented for a period of four (4) years, unless an extension is approved by the CSO following the procedure described in the CSO Decision COST 134/14 REV2.

OVERVIEW

Summary

Many trace metals (TMs) (e.g. Cu, Fe, Mn, Mo, Ni, Zn) are essential for organisms as active centres of enzymes, as about one third of all proteins are metalloproteins. Therefore, TM homeostasis in plants is at the core of many challenges currently facing agriculture and human societies. Low TM bioavailability in many soil types of large world areas causes a reduction in crop production and diminishes nutritional value of food. Some essential TMs (e.g. Cu) have narrow beneficial concentration ranges, while others (e.g. Cd, Hg) are usually only toxic, and in many areas of the world metal toxicity is a severe agricultural and environmental problem. For environmental risk assessment and remediation, as well as improved agriculture (targeted fertilisation and breeding), the mechanisms of TM uptake, distribution, speciation, physiological use, deficiency, toxicity and detoxification need to be better understood. This Action aims at elucidating them by the combined expertise of researchers (physiologists, (bio)physicists, (bio)(geo)chemists, molecular geneticists, ecologists, agronomists and soil scientists). It furthermore aims at making this knowledge applicable to the needs of farmers and consumers, with input from companies for translating laboratory results into applied products. This shall be done by integrated scientific, communication and dissemination activities, pooling together our research efforts. Regular meetings within and between the workgroups of this COST Action, training workshops for young scientists, as well as by technology transfer meetings will be organised in cooperation with the partner companies within the Action, as well as producers and merchants of micronutrient fertilisers.

Areas of Expertise Relevant for the Action	Keywords
● Biological sciences: Plant biology, Botany ● Biological sciences: Biochemistry ● Biological sciences: Biophysics ● Chemical sciences: Spectroscopic and spectrometric techniques ● Agriculture, Forestry, and Fisheries: Sustainable production	● trace metal uptake and distribution ● transition metal speciation ● trace metal deficiency and trace metal uptake efficiency ● trace metal toxicity and detoxification ● metalloenzymes and metal metabolism

Specific Objectives

To achieve the main objective described in this MoU, the following specific objectives shall be accomplished:

Research Coordination

● Progress in basic science using the synergies of expertise in the network: Determine transport pathways of metals from the soil to and through the plant; understand how plant metalloproteins are functioning; evaluate by which mechanisms metal deficiency and toxicity affect plants and how this is modulated in plant-microbe interactions.

● Develop/improve methods to achieve the scientific goals, in particular in the fields of analysing metal uptake, localisation and speciation in plants incl. determining optimal preparation methods. The Action will also set a detailed protocol to determine the metal nutritional status of plants, as well as their bioavailability for plants.

● Provide knowledge to the agricultural sector regarding localisation and bioavailability of micronutrients in plant products. Make available to farmers the best management practices for micronutrients considering crops, soils and climatic conditions. This includes agronomic approaches for reducing toxic metal accumulation in plants and providing microbial inoculants that enhance micronutrient uptake.

● Environmental and agricultural risk assessment: Evaluate the relevance of different proposed mechanisms of metal-induced inhibition of plant metabolism under environmentally relevant conditions.

● Phytoremediation of polluted soils and aquifers. This Action will improve the basis of this technology by revealing physiological and biochemical mechanisms of hyperaccumulation using the synergies of different field of expertise in the network.

● Train researchers from the public and private sectors in metal imaging and speciation methods, particularly in sample preparation, in order to avoid incorrect conclusions from artefactual metal re-distribution or changes in speciation during sample preparation.
● Provide expert advice in forums (pro and contra) dedicated to genetic modification and the use of synthetic biology in agriculture.
● Increase public awareness of the importance of trace metals in biology and their impacts on human nutrition and public health. Help industry with the technical base for the production of micronutrient-based fertilisers and authorities in their regulation.

Capacity Building
● Bridging the gap between fundamental scientists and the applied (productive, commercial) sector.
● Transferring new technologies for metal studies to the community working on trace metals in plants.
● Generating knowledge and new insights for end users to test micronutrient deficiency in crop plants, fertiliser safety and bioavailability, also in relation to the new EU regulation on fertilisers.
● Characterising and evaluating the role and safety of biological fertilisers, as well as agrochemicals, in micronutrient nutrition of crop plants.
● Developing an European-wide network of plant metallomics analyses.
● Training of young researchers through mobility promotion in academic and private sectors.

1 S&T EXCELLENCE

1.1 SOUNDNESS OF THE CHALLENGE

1.1.1 DESCRIPTION OF THE STATE-OF-THE-ART

Many transition metals (TMs) (Cu, Fe, Mn, Mo, Ni, Zn) are essential for plants and most other organisms as enzyme cofactors or structural elements. About one third of all proteins are metalloproteins, and TMs participate in almost every biological process. However, TM levels must be tightly controlled. At slightly higher concentrations than optimal ones, TM become toxic. Therefore, TM homeostasis in plants is at the core of a great number of the challenges currently facing agriculture with a profound impact on our health, food security, and social stability (Andresen et al., 2018).

Deficiency of essential TMs (in particular Fe and Zn) is widespread in large areas of the world, including major European regions. Their low bioavailability in soil handicaps plant productivity. Moreover, low TM uptake also translates to low nutritional value crops (Alloway et al., 2008). In fact, TM deficiency in human diet is one of the main causes of malnutrition in the world, causing from mild neurological and immunological alterations to death. A recent WHO press release indicates that about 5.6 million children under the age of five died in 2016, and malnutrition, especially micronutrient deficiencies (i.e., hidden hunger) has been shown as one of the major factors involved in child mortality (http://www.who.int/mediacentre/factsheets/fs178/en/). The problem may even worsen as edible parts of some staple crops contain less of essential nutrients as a response to increasing CO_2 in the atmosphere (Myers et al., 2015). Therefore, even to sustain current level of yield and to face the malnutrition problem, additional effort must be devoted to increase micronutrient levels and bioavailability in edible plant organs.

Plants critically rely on TM-dependent electron transport processes in photosynthesis and in oxidative respiration. Other uses of TMs include symbiotic nitrogen fixation, where these nutrients are provided by the host plant to the endosymbiotic nitrogen-fixing bacteria to synthesise the key enzymes catalysing the reaction. In our effort to reduce greenhouse emissions along with increased crop production we must also achieve a reduction of the use of synthetic nitrogen fertilisers, which currently feed half of the world population. As biological nitrogen fixation, either in native systems or in heterologous ones, is regaining more relevance in sustainable agriculture practices, the importance of metal uptake and delivery to nitrogen-fixing organs is now more relevant than ever (González-Guerrero et al., 2016). Moreover, TMs are not only required to optimise beneficial plant-microbe interactions, they are also an important part in plant innate immunity, either as cofactors of enzymes, or directly as part of redox or of nutritional immunity responses to plant pathogens (Aznar et al., 2015). Finally, TMs are also key elements in plant tolerance against biotic and abiotic stress, as cofactors of many of the enzymes controlling and ameliorating stress responses as well as many of those synthesising and sensing plant hormones (Andresen et al., 2018; Finkelstein, 2009). Due to climate changes, crops are more exposed to extreme weather conditions, causing difficult optimal TM uptake, which is an integral part of plant adaptation to stress. Consequently, most plant physiological processes are affected by the low metal availability, negatively impacting productivity.

Agriculture is currently struggling to meet the challenge of feeding the growing global population with nutritious food on declining available agricultural land while adapting to climate change. At the same time, the negative impact of human activities including agriculture on the environment has to be minimised. Finally, agriculture also has to adapt to anthropogenic changes that already occurred and

will continue to occur in the environment. From a metallocentric point of view, this means that under metal-deficient conditions we have to improve the uptake and physiological use of required TMs. By improving TM nutrition in plants, the Action will improve the nutritious value of crops, reduce the losses due to biotic and abiotic stress (responses mediated by metals), and by improving biological nitrogen fixation, cut in the use of nitrogen fertilisers, responsible for a large part of global greenhouse emissions. At the same time, crop plants should be improved in terms of taking up less non-essential metals in particular when enhancing metal transport, and to be more tolerant to trace metal excess toxicity, which is a widespread environmental problem to which agriculture contributes by use of metal-based pesticides and contaminated fertilisers.
In this endeavour, the Action must combine the expertise of researchers with diverse backgrounds (e.g. physiologists, (bio)physicists, (bio)chemists, molecular geneticists, ecologists, agronomists) with the needs of the farmers and biotechnological industry, with input from companies applying laboratory results into products and solutions. There is little time for non-directed approaches and missteps. New technological advances have appeared in the last decades that will greatly assist in these efforts, with better ways to visualise metals and study the molecules to which they are bound.

1.1.2 DESCRIPTION OF THE CHALLENGE (MAIN AIM)

The PLANTMETALS COST Action will tackle fundamental and applied issues related to TM deficiency or excess levels in Plant Physiology and Crop Production.

Two main approaches have been used to try to overcome the issue of TM deficiency in plants: (i) selecting new genotypes or engineering crops with improved metal acquisition and/or more efficient internal use of essential TMs (Vasconcelos et al., 2017), and (ii) use of TM-containing fertilisers (Slamet-Loedin et al., 2015; Cakmak and Kutman, 2018). However, these approaches have had limited success. To some extent, plant molecular biologists and plant breeders have developed genotypes with higher metal uptake capabilities, but the lack of biochemical information on metal transporters is often causing a parallel increase in the accumulation of non-biogenic, toxic metals and metalloids such as Cd or Hg. Similarly, there are different micronutrient-containing fertilisers on the market, but their price is relatively high and their efficiency low due to low TM availability in soil. To address these challenges fundamental scientific questions focussing on (i) the role of the plant microbiome on plant metal uptake in relation with TM chemical speciation and equilibria in the rhizosphere, (ii) metal transporter selectivity, (iii) intracellular and long distance metal trafficking, (iv) characterise the metal pools in a plant, and (v) determine how everything is regulated, must be studied. This will be translated into practical approaches that will allow us to select and develop more efficient metal transporters from germplasm, develop precision targeting of metals to assimilable fractions, as well as to identify when and in which form to apply micronutrient fertilisers and microbial inoculants. For this, methodological advances have to be made on metal imaging and speciation analysis to assist in the investigation of TM uptake, transport and localisation (Zhao et al., 2014), as well as in the identification of novel metalloproteins.

However, while striving to increase plant metal (micronutrient) uptake in some areas of the world, toxicity of various metals is a major concern in other areas because often the beneficial range of essential TMs is extremely narrow (e.g. Cu), and non-essential TMs may non-functionally replace essential metals in biomolecules (e.g. Cd replacing Zn, several TMs replacing Mg) (Andresen et al., 2018). This has many consequences for the environment as well as for agricultural use of land. Mining, smelting and industrial use (e.g. as plastic stabiliser, in pigments, batteries and other electrical equipment) increase concentrations of various TMs in the environment. The continued use of Cu-containing fungicides, even in organic farming, is strongly contributing to Cu contamination of vineyards (Pena et al., 2018) and nearby rivers and Cu-induced micronutrient deficiencies such as Fe deficiency. Along the same lines, soil Zn gradually rises due to the use of Zn as feed additive in animal production and subsequent recycling of animal manure in agriculture. The over-use of P fertilisers is known to cause Cd contamination as this metal is often a trace contaminant in them. High amounts of Cd in foods, for example in chocolates, is a growing issue in European countries, and the European Commission has set new limits for Cd in foods. To overcome these challenges, substantial advances have to be made in fundamental science including the effect of a good nutritional status on the accumulation of non-biogenic TMs (proper Zn levels minimise Cd accumulation due to their similar chemistry, for instance), improve metal transporter selectivity, and analyse TM toxicity and detoxification pathways. These results will be translated into applied approaches as improved agronomic practices, the use of novel natural or improved hyperaccumulator plants in phytoremediation (removal of toxic TMs from soils by plants) or phytomining (recovery of valuable metals from the environment by plants) alone or in combination with plant-associated microorganisms.

Considering the importance of these issues to human health, agro-business, the environment, and world policy, it is of prime interest for Europe to have fluid interactions and methods exchange between the different stakeholders in plant TM metabolism. Towards this goal, the PLANTMETALS Action will focus on TM biology in plants, in particular on TM fate in plant tissues, metalloproteins functioning, and plant-microorganisms' interactions. Attention will be also paid to impacts of beneficial metals (micronutrients) on nutritional value of food crops and agronomic approaches to improve micronutrients in crop plants. The main aim of this Action is to bring together a multidisciplinary team of researchers, agronomists, seed/fertiliser producers and farmer societies to be able to interact and collaborate towards optimal TM use by plants and how to best impact human nutrition. As a secondary goal, the Action will create a forum in which methods, approaches, and join efforts for a more thorough optimisation of plant metal nutrition. can be exchanged.

Given the importance of metals in plant biology, in the last three decades advances have been made in three main fields: (i) understanding mechanisms of TM metabolism in plants, (ii) improving micronutrient concentrations of food crops by applying plant breeding (i.e., genetic biofortification) and designing novel and targeted fertilisation strategies (i.e., agronomic biofortification). (iii) plant use for phytoremediation and phytomining.

I. TMs in plant metabolism - TM transport and distribution

Using various model organisms, the main families of metal transporters have been identified. Direction of transport and paired metal substrates have been determined mainly by phenotypical analyses in metal transport mutants and complementation in heterologous systems (like yeast). However, the biochemical basis for metal speciation and affinity for most of these families has not been identified, with the exception of some P-ATPases and COPT proteins (Andresen et al., 2018). As a result, it is not possible today to ascertain from the sequence of a transporter which metal(s) will be its most likely substrate, nor the relative affinity for a particular metal compared to others. Further, assays in heterologous systems often do not accurately reflect the in vivo situation, in particular in terms of interaction with biological metal ligands, cellular localisation, the multiple transport steps involved in root uptake, import and loading into a sink organ in a plant and the (transcriptional, translational and post-translational) regulation of transporters genes. Moreover, while some transporters for plant TM uptake from soil and loading of xylem and phloem have been characterised, the gaps in our knowledge are much bigger in the case of those that are responsible for metal loading in sink organs (young leaves, fruits, seeds/grains, tubers and legume nodules), even more so for crops that are not model crops (González-Guerrero et al., 2016).

In plants metal transporters are not the only molecules responsible for TM uptake and distribution. In fact, TMs interact with a plethora of molecules that are responsible for metal distribution and speciation (binding) in and between the organelles, saps, and across symplastically disconnected tissues. Binding to ligands and chelators avoids possible undesirable reactions (e.g. Cu is highly reactive to thiols and can displace other essential metals in proteins/cofactors). The most abundant low molecular weight molecules (organic acids, nicotianamine, amino acids, small peptides, etc.) chelating TMs have been identified (Flis et al., 2016), but it still needs to be understood how these chelators are integrated in the whole plant TM transport systems, the precise network of TM transfer, how and where speciation changes. Further, often small proteins called metallochaperones are known or assumed to be involved in inserting the metals into the active sites of proteins, but for most of the essential TMs the knowledge about these molecules is scarce.

Given the sensitivity of TMs to oxygen, their frequently low concentrations in the cell, and the wide range of metal-binding molecules, the analysis of TM localisation and speciation has not been an easy task. Only recently, with the advancement of X-ray absorption and emission spectroscopies suitable for biological samples, nanoSIMS, LA-ICP-MS and HPLC-ICP-MS, researchers have been able to study not only what metals are present, but also where they are and to some extent what is their redox state and ligand environment (Zhao et al., 2014). However, no consensus system for sample preparation and analyses exists and very few publications compare the results of different methods with the same model organism. This leads either to results that might be sample preparation artefacts, or to carrying out unnecessarily complex methods of preparation to answer relatively simple questions. For example, any damage to cellular membranes can result in metal redistribution, and to a change in speciation as well if the new environment of the metal has a different redox state, pH and/or different competing ligands than the original location. And even when a metal remains in place, the ligand environment or redox state might be changed by sample preparation or the measurement itself. In addition to the risk of producing artefacts, the lack of a comprehensive understanding is often based on the difficulties of obtaining access to all the equipment that is necessary for sample preparation and analysis. This Action will promote discussions with and between experts in the novel metal localisation and speciation studies, who will train other members of the network.

Biological roles of TMs in proteins, homeostasis and detoxification of TMs

An estimated 30% of the total proteome is predicted to be metallated (Finkelstein, 2009). Metalloproteins include electron transport chains in mitochondria and chloroplast, laccases building cell walls, zinc-finger proteins, enzymes involved in protein, nucleic acid, carbohydrate, lipid and secondary metabolism and enzymes controlling free radicals (Andresen et al., 2018). It is known for example that up to 10% of proteins in biological systems need Zn for their structural and functional integrity (Andreini et al., 2006, Clemens, 2010). However, for many more metalloproteins the role(s) remain(s) to be determined, even some whose metalloprotein nature remains unconfirmed. In summary, despite many decades of research, information on the final destination and use of TMs is still very incomplete, and many questions need to be answered to better unveil the role of metals in plant physiology.

Homeostasis of TMs regulates the uptake, sequestration, and cellular distribution of essential trace metals to have enough supply without toxic overload. Non-essential TMs need to be detoxified because they can be very toxic due to competition with essential ones and possible reactions with cellular components. Although the mechanisms of stress caused by toxic doses of TMs (in this context often referred to as "heavy metals") have been investigated for long time, often the conditions were too artificial. Usually excessive metal concentrations have been applied that rarely occur even in the most polluted environments. These high concentrations were often applied to force a toxic effect within an extremely short time (minutes to days) instead of waiting for an effect over environmentally relevant timescales (for acute toxicity and middle-term acclimation: days to months, depending on the organism). Moreover, in few studies conducted with environmentally relevant conditions and timescales, too few potentially relevant mechanisms have been investigated (due to technical limitations), so that comparison of different pathways inhibited by TM excess was impossible.

In terms of deficiency, there has been substantial research on Cu, Fe and Zn, mainly on physiological effects and changes in gene expression based on transcriptomics. What is still missing even for these metals, however, is in most cases an analysis from which level of deficiency and from which timepoint the plants really suffer from lack of key metalloproteins. This cannot be directly deduced from transcriptomics data because not all mRNA may be translated to proteins, and not all proteins will become active if the metal for the active site is missing. Therefore, an analysis by metalloproteomics in the sense of simultaneously detecting the proteins and the metals bound in them should be carried out to answer such questions. To understand TM deficiency mechanisms, transcriptomics, proteomics, and metallomics analyses need to be combined with measurements of metabolites and physiological parameters of the stressed plants. For emerging potential new micronutrients, such analyses are needed as well. This concerns e.g. Cr and rare earth elements (REE's) for which beneficial effects have been reported, but the mechanisms are not understood.

Both the response to TM excess or deficiency requires of their concentrations being determined by metal sensors. However, while some advances have been made in elucidating the mechanisms underlying the homeostasis of TMs in plants, the nature and action of the different metal sensors required remain to be identified. The number of years and the efforts invested by several research groups to unveil them with little success indicates that a concerted effort by a wider community is required.

II. Biofortification of food crops and agronomy

As indicated above, deficiency of beneficial TMs (micronutrients) in human populations is a growing concern worldwide, especially in developing countries (Muthayya et al., 2013). TM deficiencies are also reported in European countries, mainly in children and elderly people. Low amount of plant available micronutrients in cultivated soils and their low dietary intake are known as major reasons of this “hidden hunger”. Hidden hunger is associated with several health complications including impairments in immune system, brain development, mental function and physical development. Cereals, as well as root and tuber crops and pulses, represent a major source of daily calorie intake in the developing world. In rural regions of many developing countries, up to 70 % of daily calorie intake comes from cereal-based foods, which are very low in micronutrients (Cakmak and Kutman, 2018). Achieving adequate micronutrient concentrations in staple food crops represents an important global challenge and humanitarian goal. According to WHO reports, more than half of early child deaths globally is associated with conditions that could be prevented through simple and affordable interventions, such as consumption of micronutrient enriched foods. Agricultural strategies such as development of new genotypes with higher micronutrient levels through classical breeding or transgenic technologies and/or applications of efficient micronutrient fertilisers are likely the most cost-effective and sustainable solutions to the global hidden hunger issue.

Better understanding and characterisation of TM mobilisation in the rhizosphere, root uptake, root-to-shoot transport and re-translocation (mobilisation) of micronutrients into grain, for example by using high-throughput metal imaging and speciation analysis tools, would contribute significantly to on-going plant breeding programs and transgenic approaches. Mechanisms of metal acquisition and homeostasis are extremely important for breeding crops with better adaptability to changing environments and for providing healthy food with improved mineral contents. Understanding the mechanisms controlling the concentration and distribution of micronutrients (in particular Fe and Zn) in plants can give the necessary tools to increase their contents in crops but also to improve the bioavailable forms of storage. Major advances have been already obtained in the improvement of Fe content in rice grain, thanks to the better understanding of Fe homeostasis (Boonyaves et al. 2017). Combining several traits (enhancing synthesis of nicotianamine, of ferritin and engineering phytase activity) has already enabled a threefold increase in available Fe content in rice. The international HarvestPlus program has also started to release new rice and wheat genotypes with about 10 to 12 mg.kg-1 extra Zn and Fe. Efforts are on-going to achieve further increase grain Fe and Zn up to 20 to 25 mg.kg-1 to ensure impactful biological effects in the human body (Andersson et al., 2017). The combination of breeding or a transgenic approach with micronutrient fertilisation would result in additive and synergistic impacts on accumulation of micronutrients in grains at desirable levels for human nutrition. This Action will support on-going breeding and agronomic activities to maximise accumulation of micronutrients, especially Zn and Fe, for better human nutrition.

III. Phytoremediation and Phytomining

Phytoremediation can help with cleaning up polluted soils and aquifers. TM hyperaccumulators are of great interest because of their potential use in phytoremediation of contaminated soils and in phytomining, but also as models for fundamental mechanisms of metal metabolism. Recent field studies have shown that because of their enormous bioaccumulation, metal extraction per hectare and year is far higher than that of high-biomass plants with lower bioaccumulation such as corn, willows, poplars, etc. (Chaney et al., 2007). For some metal/plant combinations, e.g. *Noccaea caerulescens* for Cd, metal removal from soil has been shown to be in ranges that are already practically applicable for phytoremediation, and for some *Alyssum* species Ni phytomining has been shown to be feasible and economic, but both applications can be improved further by targeted breeding and cultivation techniques (Zhao et al., 2003; Chaney et al., 2007; Bani et al., 2015).

1.2 PROGRESS BEYOND THE STATE-OF-THE-ART

1.2.1 APPROACH TO THE CHALLENGE AND PROGRESS BEYOND THE STATE-OF-THE-ART

The PLANTMETALS Action wants to provide new knowledge and tools in the field of TM biology, with the ultimate aim of improving crops and the environment.

I. Transport pathways of TMs from the soil into and through the plant

To address the issues of TM accumulation in plants, their transport pathways will be further studied. More attention will be paid to the substrate specificity and affinity of identified transporters. How to improve Fe and Zn uptake and not accumulate Cd, Pb or Hg, will be addressed in different ways.

Through the mutagenesis of transport systems, the Action aims at reducing affinity for the non-essential competing element. Beyond classical plant models, unique plant species called hyperaccumulators will be studied. To enhance the efficiency of transport and distribution, the Action will build on published knowledge, in particular on existing databases (from transcriptomics studies). As mentioned, Fe and Zn concentrations need to be enhanced in the consumed parts of crops. More efficient transporters of Zn and Fe and different regulations of transporter genes have been shown for *Arabidopsis halleri* and *Noccaea caerulescens* (Shanmugam et al. 2011). However, characterisation of the responsible transporters is still poorly documented. This Action will address this topic. Metal compartmentation & speciation. Metal distribution will be analysed with microscopic X-ray fluorescence (µXRF), at tissue level using lab-based µXRF instruments and at sub-cellular resolution using our synchrotron collaborations. Also, the closely related techniques EDX, PIXE, or nanoSIMs can help in this task. In contrast to dyes, all these techniques based on X-ray emission can detect a metal regardless of its redox state and can detect strongly bound metal in all tissues, cells and subcellular compartments. Dyes, in contrast, usually only detect one specific redox state and only weakly bound metals. They may be useful for specifically detecting this. However, another drawback of using a dye to detect metal ions is inferior specificity compared to the X-ray based techniques. Metal stable isotope analyses via ICP-MS provide complementary insights on the pathways of TMs from the soil to plant organs since transport and chelation induce isotope fractionation. These emerging techniques have a great potential for deciphering the dynamics of TMs in the soil-plant system.

II. Other metalloproteins important for TM homeostasis, metalloenzymes

Metalloproteomics will be used to identify novel metal-binding proteins, including metalloenzymes and metalloproteins important for TM homeostasis and detoxification. The Action's participants will analyse the expression patterns and metal loading of soluble and membrane proteins by high-resolution size exclusion liquid chromatography coupled online to ICP-sfMS for the detection of metals. Hyperaccumulators will also be used to identify changes in metalloprotein properties or regulation that affect TM homeostasis and detoxification. In particular Zn and Cd hyperaccumulators that have the ability to hyperaccumulate those metals to high concentrations without adverse effects on growth, like *Noccaea caerulescens* and *Arabidopsis halleri*, will be studied. These species can be found on calamine soils (polluted with high Zn, Cd and Pb) and show remarkable adaptations to nutritionally imbalanced soils. A key property for survival on these soils is the ability of hyperaccumulator plants to maintain sufficient cellular supplies of other nutrient cations – in particular Fe – in the face of competition from other TMs (Zn, Cd and Pb) (Shanmugam et al. 2011). The mechanisms responsible for such effective metal-ion homeostasis have not yet been completely identified, but they could be of great importance in achieving a better understanding of plant nutrition and productivity.

Characterisation of metalloproteins. The Action will analyse metal affinities and their biochemical/ structural basis for important known and potentially novel metalloproteins. Further, the Action's participants will determine turnover rates of metal-dependent enzymes under different conditions, and the sequence of events and stoichiometric aspects of the transport mechanism of metal transporters. Thus, the Action's participants will investigate the mechanism of function of selected metalloproteins. Once new metal-binding proteins have been purified and identified, the genes from *Arabidopsis thaliana*, barley (*Hordeum vulgare*), soybean (*Glycine max*) will be cloned, hyperaccumulating plants or other model and crop species used by the Action's members for detailed biochemical, biophysical and structural characterisation of the proteins. Mutants will be obtained, whenever possible, from the publicly available collections and verified by PCR and sequencing. The mutants will be characterised by the methods that were already used for the wild types in order to analyse the changes in physiology.

III. Responses of trace metal metabolism to the environment: metal deficiency, toxicity, beneficial microorganisms and pathogens

The Action will analyse responses of plants to metal deficiency and toxicity by various methods, including changes in metabolite levels, secreted root exudates and whole-plant responses that are the result of direct and indirect interactions of metals with proteins, nucleic acids and metabolites. Further, better knowledge about the role of metals in plant immunity against pathogens is required. This is an exciting emerging field, since it turned out that many proteins that are essential for plant immunity need metal binding for their function. The Action will tackle these questions by the following means:

a) Metal localisation and speciation will be studied via the methods indicated in section (I.) on intact tissues, on plant extracts and in vitro assays. This will be measured in plants that have optimal compared to deficient or toxic metal supply, and in plants that are interacting with microorganisms in comparison to plants without such interactions. Thus, the Action will be able to identify and quantify changes in response to such interactions.
b) In vivo measurements of photosynthesis biophysics, metal uptake, ROS formation and respiration will be carried out. In this way the Action's participants will directly see, on a single-cell level, in how far and in which way (e.g. damage to light harvesting complexes, photosynthetic reaction centres, the electron transport chain of the Calvin Cycle) photosynthesis is affected by trace metal deficiency or toxicity stresses, and can directly correlate live metal imaging with the accumulation of metals and reactive oxygen species in the same cells. Similar assays will be carried out in plants infected by microbes.
c) Metabolomics. Like the in vivo measurements, the metabolomics analyses will help to identify target processes of metal deficiency and toxicity, so that the Action's participants want to continue them at least as an endpoint of selected experiments in parallel to subjecting samples of these experiments to µXRF, µXANES and metalloproteomics analyses. This will include starch and primary metabolites, root exudates incl. siderophores, pigments, their degradation products and other secondary metabolites, and glutathione redox state. Methodologically, it means mostly HPLC-MS and GC-MS combined with biochemical assays and UV/VIS spectroscopic methods. In addition, the Action will monitor metal accumulation on the macroscopic scale (e.g. whole leaf, whole root).
d) Metalloproteomics (HPLC-ICPMS, 2D-gels) to identify proteins involved in these interactions.

e) Plant-beneficial interactions. The plant-associated microbiome will be studied when grown under optimal and under suboptimal metal levels. Differentially associated isolates will be further analysed for their role in improving plant metal nutrition and/or protecting against toxic levels/species.
f) Plant-pathogen interactions. The effect of altered plant homeostasis in plant susceptibility to pathogens will be tested, as well as the role of metal supplementation.
g) Bioinformatics. The information obtained by the above-mentioned strategies will be combined and analysed for correlations. Causal relationships between the measured effects will be revealed and the number of candidate processes and proteins will be reduced, as false positives will be eliminated. A few examples: Physiological processes downstream of the site of action of the trace metals that were affected by metal toxicity or deficiency only in an indirect way may be identified as such if no proteins binding the respective metal are found in them. Proteins binding the trace metals as an artefact of protein isolation will be identified as false positives if they are involved in a physiological pathway/process that was found not to be affected by deficiency or toxicity of this metal.

IV. Agronomic applications

To enhance content of micronutrients like Fe or Zn in crops, this COST Action will use knowledge about plant TM biology and plant-microorganisms interactions. This will support the new global trend in targeted foliar fertilisers application on high-value crops like grapevine, strawberry or apple, to enhance natural or induced resistance and reduced fungicide use against fungal diseases like apple scab. This will have a direct impact on health of fresh fruit consumers and safer baby food production, in consent with the 2009/128/EC Directive in the sustainable use of pesticides. One important factor in the improvement of agricultural practices and crops is to determine in how far results that were obtained with the model species *A. thaliana* are translatable to crops. Therefore, this Action will also use crop plants to monitor uptake, transport and localisation of selected micronutrients such as Fe and Zn in food crops. The role of re-translocation, the stability and bioavailability of the targeted micronutrients in the processed cereal based foods will be a further research area. This Action will evaluate the potential effectiveness of TM-based fertilisers intended to enhance the long-term availability of micronutrients (Zn, Cu, Fe, Mn, Mg, Ca, Co), designed according to the new (May 2019) EU Regulation on fertilisers, which only permits selected Component Material Categories (CMCs) in fertilising products (Annex II, Part I).1.2.2 Objectives

1.2.2.1 Research Coordination Objectives

This Action focuses on achievement of these objectives:

A. Scientific

A.1 Determine the transport pathways of TMs from the soil to and through the plant. More specifically the Action aims at better understanding (i) how are TMs mobilised by plants in the rhizosphere; (ii) how do TM transport proteins specifically bind some metals and not others; (iii) how does limited TM selectivity affect TM pools and signalling in plant cells, tissues and organs; (iv) how are TM transporter function and expression regulated; (v) how do TM transport proteins interact with each other and with low molecular weight metal ligands; (vi) how are metals taken up by the roots to the shoots and distributed to and within all the sink organs of the plant (shoots, fruits, seeds but also nodules in the case of legumes); (vii) how do TM homeostatic networks interact among each other and with macronutrient homeostasis. This work should allow us to understand and improve the transport systems and the accumulation of deficient trace metals in edible parts in absorbable and useful forms, and to restrict uptake and accumulation in edible parts for usually non-essential, toxic metals such as Cd and Pb.

A.2 Understand how plant metalloproteins other than transporters are functioning. Apart from TM transporters, other metalloproteins can have an impact on nutrient use efficiency, in particular metalloproteins in which the TM plays a role in the active centre (metalloenzymes, metallopigment-protein complexes, electron-transfer proteins) or in the structure (e.g. transcription factors). Furthermore, this Action also aims at characterising novel metal-binding proteins in plants. These studies will spearhead the analyses of whole sets of metalloproteomes in biological systems.

A.3 Evaluate by which mechanisms metal deficiency and toxicity affect plants and how this is modulated in plant-microbe interactions. Such mechanisms often involve missing or wrong (non-functional) loading of metal-requiring proteins and their cofactors, but also direct or indirect interactions with nucleic acids and metabolites. All of this leads to a set of down-stream effects that ultimately cause defects in growth and fruit yield. Some of these effects occur early enough to be used for monitoring deficiency/toxicity in agricultural crops. Also, climate change plays a role in these mechanisms. Therefore, this information

will be required to select cultivars not only adapted to altered environmental conditions, but also having the highest nutritional value. Similarly, plant-microbe interactions (both beneficial and prejudicial) have an effect on plant nutrition with micronutrients. By selecting proper microbial inoculants, plant uptake of micronutrients, as well as develop policies for micronutrient fertilisation to fend-off pathogens could be improved.
A.4 Assess agronomic approaches contributing to better uptake, transport and localisation of bioavailable forms of beneficial TMs (i.e., micronutrients) in food crops. Increasing evidence supports that applications of micronutrients to soil and/or leaves greatly improve uptake of TMs such as Fe and Zn in food crops. Interestingly, in case of soil application of Zn fertilisers there is a limited accumulation of Zn in the grains. In contrast to soil applications, foliar Zn applications are highly effective in improving grain Zn as shown in wheat. Research is needed to clarify why soil Zn applications are less effective in increasing grain Zn while it works with the non-metal Se. Fe fertilisation, in contrast, is quite efficient if highly stable synthetic chelates are applied to the soil, which might be effective in improving grain Fe. However, their performance may be improved in terms of biodegradability, and in terms of chelate losses due to their low retention in the soil. This is likely not only related to soil chemistry, but also plant uptake mechanisms, with the latter being a topic of this Action. Foliar application may reduce losses and may allow the use of more environmentally friendly products, but the efficacy of foliar application of Fe products requires further studies. It is also not well-known to what extent the increased micronutrient contents in grains are still bioavailable in processed grain products such as bread. It is also important to collect new insights and data related to the inhibitory effects of a good Zn nutrition on Cd uptake and transport in plants.

B. Methodological

Where necessary, methods to achieve the scientific goals. will be developed / improved. In particular, this is expected in the fields of analysing metal uptake, localisation and speciation in plants. It would involve determining the optimal preparation methods. A detailed protocol to determine the metal nutritional status of plants/crops, as well as their bioavailability in soils and in edible parts would also be set.

C. Technology transfer

C.1 Train researchers from the public and private sectors in metal imaging and speciation methods, particularly in sample preparation, in order to avoid incorrect conclusions from artefactual metal re-distribution or changes in speciation during sample preparation.
C.2 Provide knowledge to the agricultural sector regarding the localisation and bioavailability of micronutrients in processed plant products as indicated in the outreach section below.
C.3 Make available to farmers the best management practices for micronutrients considering crops, soils and climate conditions.
C.4 Generate and share data about how agronomic approaches would be useful in reducing Cd accumulation in plants through targeted TM fertilisation.
C.5 Help farmers to identify and solve metal deficiency or toxicity in their crops.
C.6 Provide microbial inoculants, which enhance metal uptake, to the private sector.
C.7 Deposit a set of synthetic biology cassettes (genetic constructs) in modular cloning systems to deliver essential, but not toxic metals such as Cd and Hg, trace metals to specific biological processes.

Special attention will be paid to IP protection, dedicating meetings to this topic, as well as a working group (see below).

D. Outreach

D.1 Increase public awareness of the importance of trace metals in biology and their impacts on human nutrition and public health.
D.2 Help industry with the technical base for the production of micronutrient-based fertilisers and authorities in their regulation.
D.3 Environmental risk assessment. While many potential mechanisms of metal toxicity have been proposed, most of them were investigated only under artificial laboratory conditions (e.g. constant light, environmentally not relevant concentrations, etc.). It is important, for estimating actual risk, to evaluate the relevance of different proposed mechanisms of metal-induced inhibition under environmentally relevant conditions and compare the results with those obtained under conditions that were used in identifying the putative toxicity mechanisms.
D.4 Phytoremediation of polluted soils and aquifers. Hyperaccumulators of trace metals are of great interest because of their use in phytoremediation of contaminated soils, in reducing TM availability in aquifers, and in phytomining. They are important also as models for fundamental mechanisms of metal metabolism. This Action will contribute to the understanding of physiological and biochemical

mechanisms of hyperaccumulation by identifying and further characterising systems of trace metal uptake, transport and detoxification.
D.5 Provide expert advice in forums (pro and contra) dedicated to genetic modification and the use of synthetic biology in agriculture.

1.2.2.2 Capacity-building Objectives

The main capacity-building objectives of the PLANTMETALS Action can be summarised as:

1. Bridging the gap between fundamental scientists and the applied (productive, commercial) sector.
2. Transferring new technologies for metal studies to the community working on TMs in plants.
3. Generating knowledge and new insights for end users to test micronutrient deficiency in crop plants, fertiliser safety and bioavailability, also in relation to the new EU regulation on soil fertilisers.
4. Characterising and evaluating the role and safety of biological fertilisers, as well as agrochemicals, in micronutrient nutrition of crop plants.
5. Developing a Europe-wide network of plant metallomics analyses.
6. Training of young researchers through mobility promotion in academic and private sectors.

2 NETWORKING EXCELLENCE

2.1 ADDED VALUE OF NETWORKING IN S&T EXCELLENCE

2.1.1 ADDED VALUE IN RELATION TO EXISTING EFFORTS AT EUROPEAN AND/OR INTERNATIONAL LEVEL

The two COST Actions related to the present Action are completed:

CA15133: The Biogenesis of Iron-sulphur Proteins: from Cellular Biology to Molecular Aspects (FeSBioNet).15 April 2016 - 14 April 2020. This COST Action dealt with one aspect of metalloproteins but is neither focused on plants nor agriculture. Moreover, it was only considering one trace element.

TD1407: Network on Technology-Critical Elements: From Environmental Processes to Human Health Threats. 15 April 2015 - 14 April 2019. This COST Action dealt mostly with non-essential elements, such as lanthanides, and did not primarily look at their metabolism in plants or their use in agriculture.

Synergies can be established with the members of these two COST Actions, since some of the analytical tools established in these former/current COST Actions could be useful for the PLANTMETALS Action. In this direction, a joint inter-COST Meeting will be organised, for which the Action will invite members from them to a meeting.

At an international non-European level, this Action can establish synergies with on-going multinational projects such as HarvestPlus (www.harvestplus.org; www.harvestzinc.org), these projects are more on the applied level, while the PLANTMETALS Action would deliver more knowledge on the level of mechanistic knowledge. Similar approaches to those proposed above to exchange information can be set up.

2.2 ADDED VALUE OF NETWORKING IN IMPACT

2.2.1 SECURING THE CRITICAL MASS AND EXPERTISE

The breadth of the challenges indicated above cannot be properly addressed by a single group. Therefore, a multidisciplinary approach is required in which a large network bridges fundamental and applied science related to TM metabolism as well as collaboration with related industrial partners. This will go beyond an initial exchange of protocols and staff among the Action's participants to solve short-term technical problems. The combined expertise of a consortium of groups with a fundamental or applied research background with members from the private sector will allow to: (i) set common conditions to evaluate and compare the different issues studied during the COST Action, (ii) combine the expertise in several plant systems including those just used as model systems, plants studied to address specific questions (hyperaccumulators, for instance), and crops of nutritional and economical values, (iii) develop standards to determine what is "good enough" in terms of fertilisation, metal status of the plant and nutritional value, (iv) establish criteria to validate the results of the novel methodological approaches used and define the best way to employ them in both an academic and a private setting, and (v) disseminate the main findings to other members of the consortium, collaborators of the participant groups, shareholders of the companies involved, final users in the private sector, and the general public. These are tasks that no single group or even a small set of groups may carry out together; they require a network as wide as possible to broaden the scope of the questions analysed, the way to address them, and the way to use and communicate them. This will be an iterative process, sequentially improved over the different training sessions planned in the proposal and the technical sessions with the private sector. Our academic/private network will also provide mobility and training of scientific excellence to young researchers in a topical field. Thus, this Action will improve the way to address the problems of plant metal nutrition and toxicity and help to obtain the best possible outcome. Moreover, as this understanding grows, further joint collaborative Actions will be established, leading to improving plant TM metabolism and optimising its nutritional content.

The Action members are chosen to represent the field of TM metabolism in the wide sense with diverse, while intentionally often overlapping, specialisations. These include researchers who are (bio)physicists, (bio)chemists, molecular geneticists, ecologists or agronomists, as well as companies involved in agronomy, development & production of plant phenotyping and cultivation instrumentation, as well as remediation of polluted sites. Thus, a main benefit of this Action is its multidisciplinarity. This allows us to use various innovative methods, such as metalloproteomics (HPLC-ICPMS), X-ray absorption spectroscopies (EXAFS, XANES), imaging techniques using X-rays, infrared or particle beams (µXRF, nanoXRF, µFTIR, nanoSIMS, µPIXE)), stable isotope analyses (after labelling or at natural abundance), transcriptomics via next-generation sequencing, metabolomics, etc. the PLANTMETALS Action will establish a pan-European platform for metal localisation and speciation analysis in plant samples. Moreover, the combined expertise will lead to the next step in metallomics, which is identifying the metalloprotein and metal-chelate complement of a plant, towards unveiling their role in plant physiology. Besides using existing methods, the interaction of scientists within this Action will furthermore help in improving methods of sample preparation for these techniques and application of these techniques.

Gender balance will be promoted in tasks involving leadership, in the scientific exchanges and participation in summer schools. A main emphasis will be put on the training of young researchers, on their mobility inside the network to learn new tools and to train in the private sector, as well as in training private sector employees in the novel metallomics methods available at fundamental research institutions.

2.2.2 INVOLVEMENT OF STAKEHOLDERS

In scope of this COST Action, dissemination activities will be organised to help involve all relevant stakeholders and achieve three main dissemination goals: (i) to share our research results and outcomes; (ii) to stimulate the construction of new research projects; (iii) ultimately, to influence decision making and to raise awareness to the topic of mineral nutrition and sustainability of farming systems. The most relevant stakeholders are: (i) scientists of the public and private sectors, (ii) national and regional agriculture and environmental agencies and regulators, (iii) farmers, (iv) industrials, (v) students in Bio-related areas (Biology, Biotechnology, Agronomy, etc.), and (vi) general public. Representative European members of the first four types of stakeholders are participating in this Action and would also attract additional partners from their respective networks. The involvement of students will be obtained through incorporating some of the topics of this proposal in the undergraduate curricula, as part of the teaching duties of the participating researchers, with summer schools, and by offering internships. General public will be involved through outreach activities and detailed information on the outcome of the Action through an online approach (web page, social networks, press releases, educational material, etc.).

The Action's plan to maximise its impact includes: (i) good management, taking care of the interactions with the different stakeholders, (ii) broad communication through all media, specialised web sites, publication in open-site scientific journals, summer schools, (iii) invitation of different stakeholders at annual scientific meetings, (iv) active exchanges between participating laboratories (of researchers and of material), and (v) dynamic web-sites with open sections on analysis methods, collaborations, etc., and an active presence in social networks.

To further ensure the impact on the general public and farmers, targeted approaches will be used consisting of: (i) press releases after major discoveries will be directed to news agencies and to broadcasting programs interested in biotechnology/agriculture (for instance Onda Agraria in Spain, ARTE in France), as well as distributed via social media such as twitter and Facebook (ii) technical notes in farmer bulletins, and (iii) participation in seminars, roundtables, meetings, fairs, exhibitions directed to the productive sector (e.g. FruitAttraction in Spain, Paris International Agricultural Show, etc.).

2.2.3 MUTUAL BENEFITS OF THE INVOLVEMENT OF SECONDARY PROPOSERS FROM NEAR NEIGHBOUR OR INTERNATIONAL PARTNER COUNTRIES OR INTERNATIONAL ORGANISATIONS

The PLANTMETAL Action initially comprises 56 research groups, and 4 companies, covering a multidisciplinary approach to the study and use of TM biology in plants. For specific technical issues, the Action shall use the wide network of international collaborations of the Action’s participants. In addition, by the beginning of the Action, recommendations will be targeted to international organizations such as FAO, or WHO, likely mediated by the participation of some of the Action’s participants.

3 IMPACT

3.1 IMPACT TO SCIENCE, SOCIETY AND COMPETITIVENESS, AND POTENTIAL FOR INNOVATION/BREAK-THROUGHS

3.1.1 SCIENTIFIC, TECHNOLOGICAL, AND/OR SOCIOECONOMIC IMPACTS (INCLUDING POTENTIAL INNOVATIONS AND/OR BREAKTHROUGHS)

The short-term scientific impact of this Action will be a broader and improved collaborative interaction between European researchers working on different aspects of TM metabolism in plants. The long-term consequence of this improved collaboration is a higher productivity of research groups, higher mobility and better training of young researchers in this challenging research area, faster achievement of goals, more applicable outputs useful in everyday agriculture, and more efficient use of research money.

Technologically, based on the improved scientific collaboration, the PLANTMETALS Action will enhance the use of high-end research infrastructure such as beamlines for analysing metal distribution and speciation, HPLC-ICPMS installations, etc. It will furthermore improve the consistency and reliability of how samples are prepared for such analyses, and thus diminish the production of artefactual results.

Socioeconomically, via knowledge transfer in various ways described above it will lead to improved crop breeding and fertilisation in terms of micronutrient metabolism, better environmental risk assessment and mitigation strategies. As a result of these advances, the scientific outcome has also the potential to create new biotechnology companies and jobs related to the better TM crop nutrition as well as lower load of toxic metals spread in food and the environment.

3.2 MEASURES TO MAXIMISE IMPACT

3.2.1 KNOWLEDGE CREATION, TRANSFER OF KNOWLEDGE AND CAREER DEVELOPMENT

A Management Committee (MC) will be created with a Chair, a Vice-Chair, Working Group (WG) Coordinators (2 coordinators per WG from different participating countries), and Short-term scientific missions (STSM) coordinator. To foster the next generation of leaders in the field of TMs in plant biology, where possible one of the two WG coordinators will be an early career investigator, elected among the participating ECIs. The already achieved strong participation of renowned female group leaders in this proposal will set the foundations of gender equality in the managing structures. Moreover, a mentoring network and a “Women in plant biology” session will be included in COST meetings to nurture the next batch of female scientists in our community.

MC meetings will take place once a year, during which the different WG meetings (once a year for each WG) will be decided. The tasks of the two WG co-ordinators will be:

i. to organise the scientific meetings,
ii. to coordinate the activities (exchanges, summer-school, teaching, etc.),
iii. to promote the set-up of joint research projects,
iv. to report on the progress to the MC and help promote communication,
v. to participate on the yearly MC meetings.

During WG meetings,

i. progress will be assessed,
ii. annual progress reports will be produced,
iii. exchanges of researchers between labs will be fostered (with the help of STSM financial support) and past STMS will be evaluated,
iv. exchanges with industry and training of researchers in industry will be organised,
v. the website will be updated.

When appropriate, co-WG meetings will be organised. During single or co-WG meetings, the integration of the different expertise, of public/private sectors, in multidisciplinary works will be stimulated. Projects on common themes, currently fragmented at the national level, will be progressively integrated and European projects will be encouraged. Whenever appropriate, inter-COST workshops will be organised (e.g. other COST Actions on plant/micro-organisms interactions, nitrogen fixation).

Training of young scientists will be organised by (i) exchanges financed by COST (Short-Term Scientific Missions = STSMs) or with other financial support; for e.g. EMBO Short-Term Fellowship, (ii) FEBS Short-Term or Summer Fellowship, (iii) MSCA Individual Fellowship, (iv) summer schools (e.g. sample preparation for metal analysis, metallomics), (v), teaching activities, (vi) internships in industry. For career development, this Action will provide and disseminate job opportunities to the stakeholder network. It will disseminate available and upcoming research opportunities/funding programmes, which could help the long-term sustainability of the PLANTMETALS. Action.

Mobility will be promoted through travel grants, with a focus on travel to and from ITC teams.

3.2.2 PLAN FOR DISSEMINATION AND/OR EXPLOITATION AND DIALOGUE WITH THE GENERAL PUBLIC OR POLICY

A website containing useful information (memorandum of understanding, announcements of events/meetings/summer schools/open days, articles and relevant information in media, progress reports) will be created. The website will also announce job vacancies, possibilities of trainings and summer schools.

Knowledge and data from this COST Action will be presented at international conferences for promoting European know-how and increasing international collaborations.

Publications of original results and of reviews will be fostered in international peer-reviewed journals. In particular, highlights presented at Working Group meetings will be communicated to specialised sections in journals (e.g. New Phytologist, Plant Physiology, Plant and Soil, etc.) with the agreement of the speakers. Publication of specialised books will be welcome if and when appropriate.

Teaching activities, summer-schools for PhD students and/or post-graduates will be yearly organised (and announced on the website).

Dissemination to the public is another major issue. Special efforts and initiatives will be undertaken at the local level in the participating universities/institutes and NGOs. Dissemination to the general public, policy makers and stakeholders will be done through appropriate direct contacts, the media incl. social media like Facebook and Twitter, organisation of open days (EPSO Plant Days, Fascination of Plants Days, World Food Days, European Researchers' Nights, etc.), and seminars at the national level. In selected European countries, meetings could be organised dealing with the environmental, crop production and human nutritional aspects.

Technology transfer meetings will be organised in cooperation with the partner companies within the Action, as well as with producers and merchants of micronutrient fertilisers, to facilitate the transfer of the knowledge gained from the COST Action.

4 IMPLEMENTATION

4.1 COHERENCE AND EFFECTIVENESS OF THE WORK PLAN

4.1.1 DESCRIPTION OF WORKING GROUPS, TASKS AND ACTIVITIES

The PLANTMETALS Action is divided into six working groups (WGs). The first four WGs correspond to the scientific objectives, while WG5 and WG6 address capacity-building, intellectual property and outreach.

WG1 (Metal transport): Pathways of TMs from the soil and through the plant. WG1 will identify the limiting factors in the uptake of plant physiologically active TMs from the rhizosphere (task T1.1) and limit the uptake or the transfer to edible parts of toxic non-essential TMs (T1.2) WG1 will determine the chemical form (speciation) of TMs in each compartment (T1.3). WG 1 will study crop plants and the use of hyperaccumulators and identify key nodes to improve/limit the uptake and accumulation of essential/non-essential TMs (T1.4). WG1 will study interactions between TMs (antagonistic or synergistic effects) (T1.5).

WG2 (Metalloproteins): Metalloproteins important for TM homeostasis, metalloenzymes. WG2 will identify metalloproteins important for TM use efficiency (task T2.1). WG2 will study interaction of the proteins (other than transporters) with a TM: substrate affinity, regulation of activity, mechanism of function (T2.2).

WG3 (Environment): Responses of plant TM metabolism to the environment: TM deficiency, TM toxicity, interactions with beneficial microorganisms and pathogens. WG3 will identify targets of both stress conditions and mechanisms, from the initial target to the whole- plant response (task T3.1). The initial targets can be lack or non-functional replacement of metal centres in metalloproteins, but also direct or indirect interactions with nucleic acids and metabolites. WG3 will reveal how beneficial microorganisms and pathogens interact with the metal metabolism of plants (T3.2). Such interactions may, for example, involve changes in the uptake and intra-plant distribution of metals, enhanced or diminished expression or activity of metalloproteins.

WG4 (Agronomy): Agronomic aspects of TM homeostasis. WG4 aims to conduct studies to contribute to better nutritional quality of food crops by using targeted fertilisation and agricultural management also in consideration of the new EU regulation on fertilisers (task T4.1), and to characterise crop plant response to low and excess application of TMs by employing physiological assays and analytical tools, which will be developed in WG1-3 (T4.2). WG4 will evaluate how micronutrient fertilisers are absorbed (after foliar vs. soil application, added in organic or mineral forms) (T4.3), how microorganisms and fungi can be used to improve plant TM nutrition (through a better use of soil metals) (T4.4), how TMs are transported within crop plants and how their localisation and speciation changes in this process (T4.5). Finally, workshops / symposia will be organised for farmers associations and industrial stakeholders dealing with targeted breeding, fertilisation and micronutrient foliar fertiliser production (T4.6). This will be done in the last year of the Action, to facilitate the transfer of the knowledge gained from the COST Action to the stakeholders.

WG5 (Dissemination): Dissemination. WG5 will be focused on how to best target the results from this Action to the different stakeholders (task T5.1). WG5 will be responsible for preparing the press releases to news outlets (T5.2), update the Action's social media accounts, and support the efforts at the national front. Moreover, WG5 will organise training sessions on writing skills to target research journals, farmer bulletins, and press releases (T5.3). In addition, WG5 will coordinate any joint project by groups within the consortium to maximise group interactions, avoid overlaps and redundancy of research efforts, and increase success rates (T5.4).

WG6 (IPP): Intellectual property protection. It is expected that a number of the results obtained within the PLANTMETALS Action will have an impact on the private sector. WG6 will coordinate all the IP protection efforts (task T6.1), promoting them whenever possible in collaboration with the universities involved – (which usually have their own IP department) (T6.2). In addition, to maximise this output, WG6 will organise a training session on IP protection for the younger participants (T6.3) and promote meetings with the private sector to ensure the exploitation of the key results of the Action (T6.4).

WG1-6 will have strong and synergistic interactions and dialog as outlined in Figure 1. These interactions will be further promoted by the general meetings of the COST Action to which meetings of the individual WG will usually be linked (see Gantt diagram in 4.1.4), by Short Term Scientific Missions (STSM's) and the other activities described in detail in section 3.2.

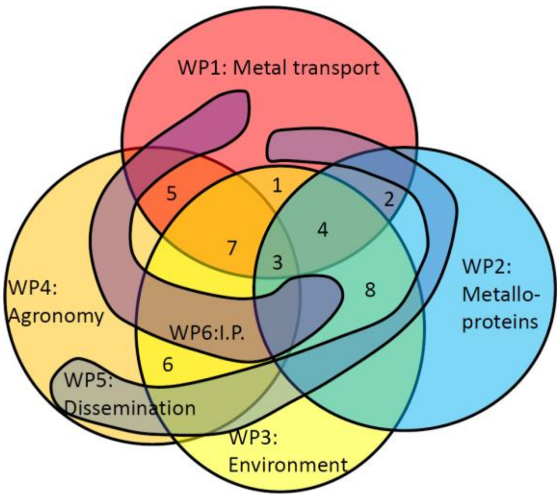

Figure 1. Interactions between WPs in the PLANTMETALS Action. (1) Metal solubilising microorganisms in nature; (2) Metal loading of the active centres / cofactors of metalloproteins; (3) Interactions between siderophores mediating metal uptake, metal solubilising microorganisms and plant systemic responses to microbes; (4) Metal detoxification pathways / Metabolic processes affected by metal levels in the environment; (5) Modification of metal accumulation in crops by changing metal transporter expression; (6) Microbial inoculants for metal nutrition / Crop biofortification; (7) Improved metal uptake from soil and delivery to sink organs; (8) Metal toxicity by non-functional substitution of active centres / metal cofactors in metalloproteins. WG5-6 are made up of members of WGs 1-4. WG5-6 members will therefore be automatically present at the activities of the scientific WGs while they will ensure the dissemination and IP objectives.

4.1.2 DESCRIPTION OF DELIVERABLES AND TIMEFRAME

One of the key aims of this Action is to bring together the wide range of perspectives and biological questions relating to transition metals in plant physiology, create a forum in which the greater European community working in the field can interact and collaborate. As deliverables, major advances in the following fields, described together with the expected timeframe, should be expected.

Methods. By joining the major European experts in TM visualisation and speciation (incl. small ligands and metalloproteins), in the first year we will develop a standardised protocol for metallomics analyses in plants and associated microorganisms. PLANTMETALS should also enable the creation of a multi-institutional platform, or an advisory body, to better direct researchers to the proper metallomics facilities and methods to target specific biological questions while optimising European resources. Throughout the duration of the Action, combined expertise will also lead to novel technological developments in metal imaging by X-rays, mass-spectrometry approaches to metallomics, and analytical biochemistry of metal complexes. There is limiting availability of beamtime at synchrotron facilities, and the limiting number of existing platforms with expertise in HPLC-ICPMS or analytical biochemistry. This will be overcome by: (i) training sessions on beamtime proposal writing will be organised to improve beamtime access, (ii) the use of in-house beamlines where the high resolution of synchrotrons is not required, (iii) training sessions will be organised to train new experts in analytical approaches to metallomics, and (iv) proposals will be made by the consortium to expand the number of EU-supported facilities for metallomics analyses, as well as assist in applications to national programs by the participating members. This should not only increase, on the long run, the number of places to carry out these experiments, but to better rationalise their use already from the start of the Action.

Fundamental science. The joint efforts of the participating laboratories will provide new insights on how plants deliver TMs to the sink organs, what are the beneficial and toxic interactions of these TMs with plant metabolism, and how plants defend themselves against deficiency and toxicity. This will involve the identification and characterisation of new metal transporters, metal-binding proteins and metalloenzymes. The Action will also contribute to the characterisation of the role of the microbiome on plant metal nutrition, and tolerance to toxic levels, as well as how plants use metals to combat pathogens. All these lines are frontier research that will be greatly promoted by the technical advances described above, as well as from the insight of the consortium members. Some of these studies are quite straightforward (e.g. analysis of mutants, phenotypic assays) and likely to succeed in the first half of the Action. Other, such as the biochemistry of new metal transporters or metalloenzymes might be more difficult and will take longer. These will be addressed using the combined expertise of the consortium, in which the number of technical approaches will be greatly expanded, combining the classical genetic complementation assays with protein biochemistry and structural and computational analyses. Similarly, problems in the characterisation of microbial communities in a particular crop/soil combination will be solved better by the combined expertise of the consortium, which can provide access to computing facilities for improved analyses of plant microbiomes.

Applied Science. The use of several different experimental models will allow us to identify commonalities and peculiarities of TM biology in crops. As the final outcome at the end of the Action, this should lead to improved breeding strategies to enhance the nutritive value of crops in the current context of climate change, as well as reduced environmental pollution by agriculture. In collaboration with private institutions, and using state-of-the-art metal imaging methods, from the beginning of the Action should be able to contribute to new methods for improving plant metal uptake. Taking advantage of the combined expertise on phytoremediation and on tolerance to toxic metals, the Action should be able to help breeders and farmers to increase the content of essential TMs in crops where they are deficient, while diminishing the uptake of TMs when they are in excess (incl. essential TMs) or generally toxic (e.g. Cd, Hg). Similarly, the knowledge generated by this Action should help improve phytoremediation via targeted breeding (long-term) and improved agricultural management (applicable already within the Action duration). One problem, which will have to be addressed from the beginning and throughout the Action, is the risk of increasing the levels of toxic TMs by improving uptake of essential ones. To solve this is already one of the core activities of the consortium, for which metal selectivity in plant metal transport will be addressed. In addition, the studies on the metalloproteome of modified plants, and their intracellular TM transport will provide new strategies to direct needed metals to the correct compartments/proteins, while preventing toxic metal accumulation.

However, these are merely the foreseeable breakthroughs. Given the array of participants from very different areas of plant physiology and agronomy, novel unforeseen results should be expected.

4.1.3 RISK ANALYSIS AND CONTINGENCY PLANS

Risk 1: The main risk in PLANTMETALS is that some partners might not be able, e.g. because of funding problems for the actual research or unexpected time-consuming duties, to collaborate as much as intended at the time this proposal had been written. These potential risks are minimised in by the large size of the network sharing overlapping fields of expertise, so that others can fill the eventual gaps if needed. To prevent lack of coordination, WP participants will send a semesterly short report (1 page) on the main experiments and developments in their groups to the WP leaders. Should redundant efforts be detected, a meeting with the involved parties would be held to improve synergies to optimise the work and avoid competing efforts.

Risk 2: Another main risk is to have a lack of time to develop all the tools within the timeframe of the proposal. This will be addressed by an early kick-off meeting to frame the problems and with continuous communication with the Management Committee, between the WP leaders and the WP members to prioritise efforts and optimise collaborative efforts.

Risk 3: An additional risk for a big network is that the number of collaborative visits may become too large for the Action budget and unforeseen political/safety problems might jeopardise planned meetings. In both cases, the use of multimedia allowing to set up webinars and videoconferences will help to replace some of the physical travelling and meeting. This will also help to reduce the carbon footprint of this Action.

Risk 4: Finally, there can be unpredicted issues. In this sense, the existence of a wide network of researchers, such as the one forming this Action will ensure that the expertise is present to overcome any difficulty.

4.1.4 GANTT DIAGRAM

All dates written below have to be kept somewhat flexible to be able to deal with unforeseeable developments of the Action. For this reason, the years are already only divided into quarters, but a move between them may still be possible.

	Year 1				Year 2				Year 3				Year 4			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
General activities																
	CGM						CGM				CGM					CGM
	ICM															
Work group activities																
WP1 Metal transport	WGM		TWS				WGM				WGM					WGM
WP2 Metalloproteins	WGM		TWS				WGM				WGM					WGM
WP3 Environment	WGM		TWS				WGM				WGM					WGM
WP4 Agronomy	WGM					WGM				WGM				TWS		WGM
WP5 Dissemination	WGM	TWS					WGM				WGM					WGM
WP6 IPP	WGM	TWS					WGM				WGM					WGM

Milestones

CGM: COST Action general meetings, incl. the MC and all WGs. CGM's are milestones for all WPs as they mark the inauguration of PLANTMETALS (year 1), coordination and verification of the progress (year 2 and 3) and the evaluation of the final outcome (year 4).
ICM: Inter-COST meeting with representatives of other COST Actions, for optimising collaborations.
WGM: Workgroup meetings: They act as milestones like the CGM's, but specific for individual WPs.
TWS: training workshops (Including summer schools) for young scientists, farmers, etc. and representatives of multinational projects such as HarvestPlus. They are milestones for the transfer of knowledge to key stakeholders within and outside of the PLANTMETALS network.

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