Interreg
North-West Europe
NWE-REGENERATIS
European Regional Development Fund

13 September 2022

DI2.3.3 Greenhouse demonstration for vegetal production for ecocatalyst synthesis

Adeline JANUS[1], Julien BERNY[1], Tristan DEBUIGNE[1], Gaëtan THIBAUT[1], Antoine WENDERBECQ[1], Alina GHINET[2], Christophe WATERLOT[2]
[[1]IXSANE, [2]JUNIA]

Version 1

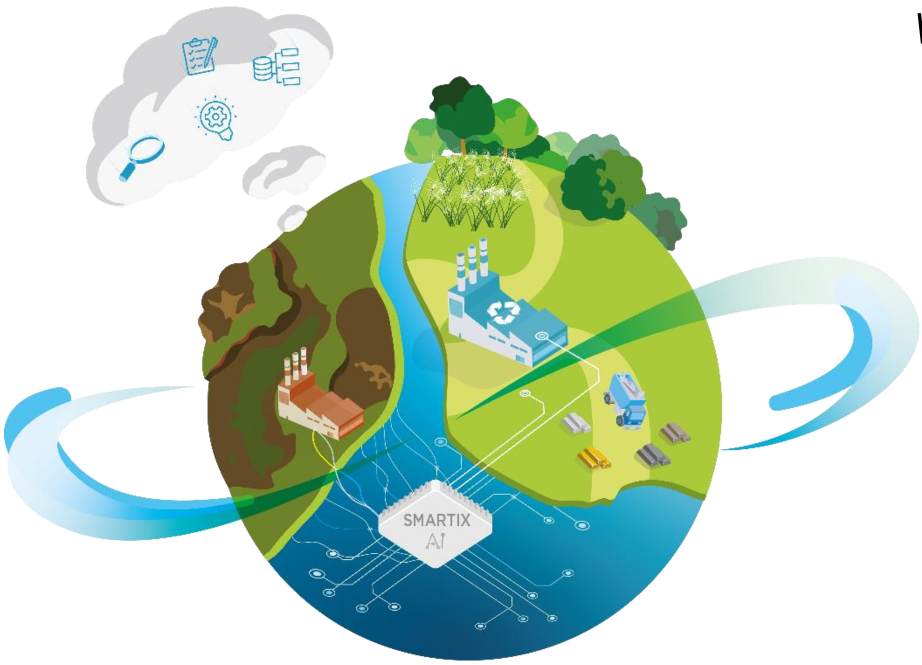


TABLE OF CONTENT

List of Figures

List of Tables

EXECUTIVE SUMMARY

In the REGENERATIS project, one of the objectives is to produce ecocatalyst from past metallurgical sites and deposits. In the report WPT2.3.1 untitled "Technical report on lab test undertaken for remaining metallic contamination management in soil", the first steps of the methodology have been employed. It consisted of selecting several soil samples (< 1 kg) from several sites based on the type of heavy metals, their contamination level, and the fertility of soils to evaluate the potentiality of growing and development of ryegrass on these soils.

The pilot site concerned in this deliverable is the French site, called POMPEY site, and owned by EPF Lorraine. The site has been characterized in terms of fertility and metal contamination and it allowed to select two soils highly contaminated by Zn (called P1 and P2) for the next greenhouse experiment.

The experiment consisted in evaluating the transfer of metals from the soils to ryegrass and its interest to produce ecocatalysts. Thus, we experimented 2 soils from the site but also two different amendments: bone ash and hydroxyapatites. The objectives were: i) to compare the different soil tested, ii) to evaluate the effectiveness of the amendments, iii) to measure the transfer of metals to ryegrass, iv) to produce efficient ecocatalysts.

With the French soils, the amendment has no or low effect on soil parameters and metal availability. The ryegrass biomass was quite high as well as the concentrations of Zn in the aerial parts. Based on these results, the efficiency of ryegrass produced on the two soils will be evaluated to produce ecocatalysts.

The results of this greenhouse experiment can also be found in the deliverable WPT2.3.2 'Report on lab pilot scale tests (1-25kg) for soil fertility treatment & ecocatalyst production potential undertaken on samples" where these results are also compared with those obtained with another pilot site located in Belgium.

1 INTRODUCTION

In the REGENERATIS project, one of the objectives is to produce ecocatalyst from past metallurgical sites and deposits. In this context, the ryegrass *Lolium perenne* L. was selected to extract metals of interest from contaminated soils by heavy metals.

In the report WPT2.3.1 untitled "Technical report on lab test undertaken for remaining metallic contamination management in soil", the first steps of the methodology have been employed. It consisted of selecting several soil samples (< 1 kg) from several sites based on the type of heavy metals, their contamination level, and the fertility of soils to evaluate the potentiality of growing and development of ryegrass on these soils.

The pilot site concerned in this deliverable is the French site, called POMPEY site, and owned by EPF Lorraine. The site has been characterized in terms of fertility and metal contamination and it allowed to select two soils highly contaminated by Zn (called P1 and P2) for the next greenhouse experiment.

The experiment consisted in evaluating the transfer of metals from the soils to ryegrass and its interest to produce ecocatalysts. Thus, we experimented 2 soils but also two different amendments: bone ash and hydroxyapatites. The objectives were: i) to compare the different soil tested, ii) to evaluate the effectiveness of the amendments, iii) to measure the transfer of metals to ryegrass, iv) to produce efficient ecocatalysts. The results of these experiments will be described hereafter.

2 Materials and methods

2.1 Soil characteristics

As described in the report WP T2. D3.1 "*Technical report on lab test undertaken for remaining metallic contamination management in soil*", two soils from the Pompey site have been selected for lab experiment (P1-A and P2-A). The physicochemical characteristics of the two soils are described in the **Tables 1** and 2.

The two soils present a basic pH and high $CaCO_3$ content, a good organic matter content, a low nitrogen content and a poor CEC. P1 and P2 are highly contaminated by Cu, Hg, Pb and Zn. Moreover, the sample P1 is also contaminated by As and Cr. The two soils present a very high contamination by Zn.

Based on the results, the samples P1-A and P2-A have been selected for the next experiments with the aim at evaluating the development of ryegrass and the transfer of metal from the soil to the aerial biomass to produce ecocatalysts.

Table 1: Agronomic parameters of the soils from Pompey

Sample	Texture	pH	$CaCO_3$	OM	total N	C_{org}/N_{total}	CEC	Electrical conductivity	Available P
		-	*$g\ kg^{-1}$*	*$g\ kg^{-1}$*	*$g\ kg^{-1}$*	-	*$cmol^+\ kg^{-1}$*	*$mS\ cm^{-1}$*	*$g\ kg^{-1}$*
P1-A	Sand	7,7	199	91	2,80	18,9	27,1	0,21	0,135
P2-A	Loamy sand	8,1	201	60	2,01	17,4	34	0,85	0,094

fertility = 0/3 ; fertility = 1/3 ; fertility = 2/3 ; fertility = 3/3

Table 2: Concentration of metal(loid)s in the two soils from Pompey

Sample	Sb	As	Ba	Cd	Cr	Cu	Hg	Pb	Mo	Ni	Se	Zn
	$mg\ kg^{-1}$ DW											
P1-A	41	140	1400	*<68*	110	130	14	6 900	<10	77	<5	11 000
P2-A	54	42	2500	*<160*	68	140	5,9	13 000	<10	22	<5	21 000

[metal] > threshold value (SPAQUE, 2004) [metal] > intervention value (SPAQUE, 2004)

2.2 Soil amendments

Two amendments were tested in this experiment. The first amendment is bone ashes and the second is hydroxyapatites. These amendments were applied at different rates according to the concentrations of Cd and Pb in the soils. Indeed, the objectives of these amendments is to fix metals considered as undesirable for ecocatalyst production in the soil (e.g. Cd, Pb) while allowing the transfer of desirable elements from the soil (Zn) to the aerial biomass of the plant.

Thus, to determine the amendment rates, we evaluated the concentrations of Cd and Pb in the soils and the sorption percentages of Cd and Pb on the amendments (according to the scientific literature). The calculations of the amendments rates for the soils from POMPEY are described in **Table 3**.

NB: For the POMPEY soil, we evaluated the available concentrations of Cd and Pb (with acid acetic extraction) due to the very high total concentrations of metals in soils.

Table 3: Calculation of the amendment rates for the Pompey soils

Site	Sample reference	Total Pb	Available Pb	Total Cd	Available Cd	Total	Cd/Pb adsorption	Quantity of bone meal ash / hydroxyapatite	Amount of soil	**Quantity of bone meal ash / hydroxyapatite**	*Amendment*
		mg kg^{-1}	*mg kg^{-1}*	*mg kg^{-1}*	*mg kg^{-1}*		*%*	*g kg^{-1}*	*kg*	***g pot^{-1}***	*%*
POMPEY	P1	6900	105	<68	8,7	113,7	5	2,3	1,5	**3,4**	*0,23*
	P2	13000	69	<160	7,8	76,8		1,5		**2,3**	*0,15*

2.3 Experimental design

The two amendments were applied to the soils according to the amendment rates defined previously. After amendments, the containers were placed in a greenhouse for 1 month for equilibrium and maintained to constant humidity with tap water. An unamended soil was used as control.

At the end of the equilibrium period, the soil was mixed and 1.5 g of ryegrass / kg of soil (*Lolium perenne* L. var Cantalou) was sown on the soil surface. During the experiment, the pots were regularly humidified with tap water to maintain a humidity adequate for ryegrass cultivation (**Figure 1**).

Figure 1: Ryegrass development with soils from POMPEY

After 8 weeks, the ryegrass shoots were harvested by cutting 0.5 cm above the soil with scissors. They were weighed and washed three times with tap water and osmosed water to remove any dust particles. The samples were oven-dried at 40°C for 48 h and weighed once again to determine the fresh and dry biomass. Finally, the samples were ground into fine powder using a knife mill.

Once again, the pots were regularly humidified with tap water for 8 weeks to allow a second crop of ryegrass. Then, ryegrass was cut and treated as the first crop. Roots of ryegrass were also sampled from the soils. Then, the soils were air-dried, ground, and passed through 2-mm and 250-µm sieves using a mill.

The concentrations of metals in the ryegrass shoots and roots were determined by atomic absorption spectrophotometry after an acidic digestion with HNO_3 and H_2O_2. The concentrations of metals in soils were determined after an acidic digestion in aqua regia (HCl and HNO_3). Available phosphorus, pH and $CaCO_3$ content of the soils were also evaluated.

3 Results

3.1 Soil

Table 4 presents the chemical characteristics of the soils from POMPEY.

Table 4: Chemical characteristics of the two soils from POMPEY

				Pseudototal			
Sample reference	pH	$CaCO_3$	P_2O_5	Cd	Cu	Pb	Zn
		g kg^{-1} DW	g kg^{-1} DW	mg kg^{-1} DW	mg kg^{-1} DW	mg kg^{-1} DW	mg kg^{-1} DW
P1 - Unamended	7,98 *0,01*	168 *8*	0,107 *0,003*	36,0 *4,3*	149 *8*	12 770 *1 720*	11 379 *763*
P1 - Bone ash	8,02 *0,03*	174 *2*	0,111 *0,004*	32,5 *1,4*	144 *8*	10 263 *313*	10 266 *531*
P1 - Hydroxyapatites	8,05 *0,04*	168 *1*	0,143 *0,03*	32,8 *3,0*	149 *9*	11 241 *2 921*	10 391 *936*
P2 - Unamended	7,97 *0,03*	148 *3*	0,069 *0,008*	85,5 *6,4*	114 *11*	17 710 *681*	22 558 *1 207*
P2 - Bone ash	8,12 *0,01*	157 *6*	0,067 *0,007*	91,4 *8,9*	105 *3*	17 302 *729*	23 878 *1 273*
P2 - Hydroxyapatites	8,03 *0,02*	158 *8*	0,075 *0,012*	86,5 *2,5*	103 *8*	17 433 *1 193*	28 798 *12 902*

The pH for the soil P1 is ranged between 7.98 and 8.05 and between 7.97 and 8.12 for the soil P2, revealing basic soils with no effect of the amendments. Carbonates content for the soil P1 is ranged between 168 and 174 g kg^{-1} DW and between 148 and 158 g kg^{-1} DW for the soil P2, revealing a higher carbonates content for the last soil. The amendments had no effect on this parameter.

Differences are observed between the conditions for available phosphorus (**Figure 2**). The soil P1 has higher P_2O_5 content (between 0.11 and 0.14 g kg^{-1} DW) than the soil P2 (between 0.07

and 0.08 g kg^{-1} DW). Moreover, the input of hydroxyapatites in the soil P1 increase the content of available phosphorus by 34 %. However, this increase was not observed for the soil P2.

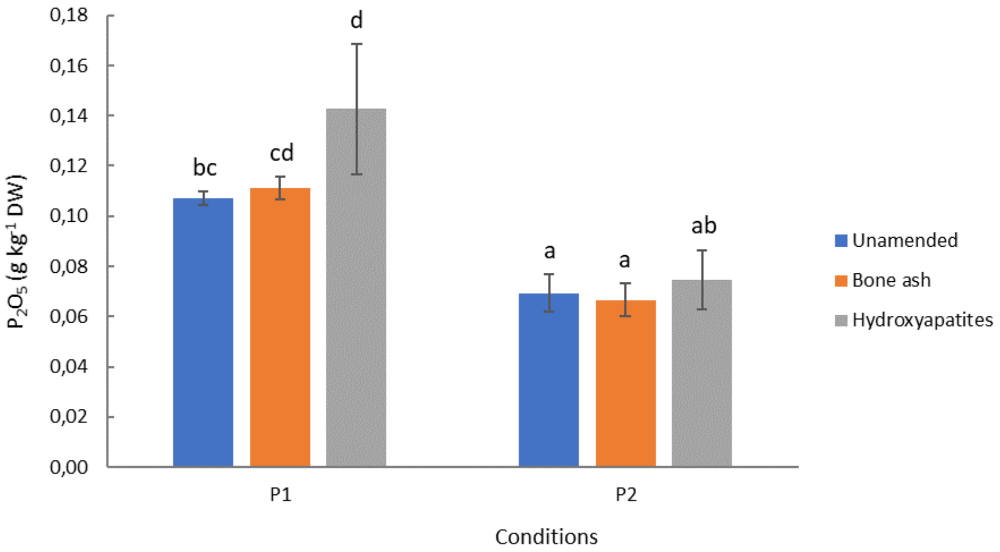


Figure 2: Phosphorus concentrations (g kg^{-1} DW) in soils from POMPEY

3.2 Ryegrass

3.2.1 Biomass

The aerial biomass of ryegrass was evaluated for all conditions for the first and second cut (**Figure 3 and 4)**. At the first cut, the fresh biomass ranged from 50.3 to 63.2 g and the dry biomass ranged from 6.5 to 9.0 g. At the second cut, the fresh biomass ranged from 22.6 to 33.3 g and the dry biomass ranged from 5.0 to 8.0 g. Statistical analyses revealed no effect of the amendments. However, at the first cut, a significantly higher dry biomass was observed for the soil P2 compared to the soil P1. This trend was also observed at the second cut but was not statistically significant. Finally, we observed a high decrease of ryegrass biomass between the first and the second cut. This result could be explained by a depletion nutrient resource in the soils leading to a lower ryegrass growth.

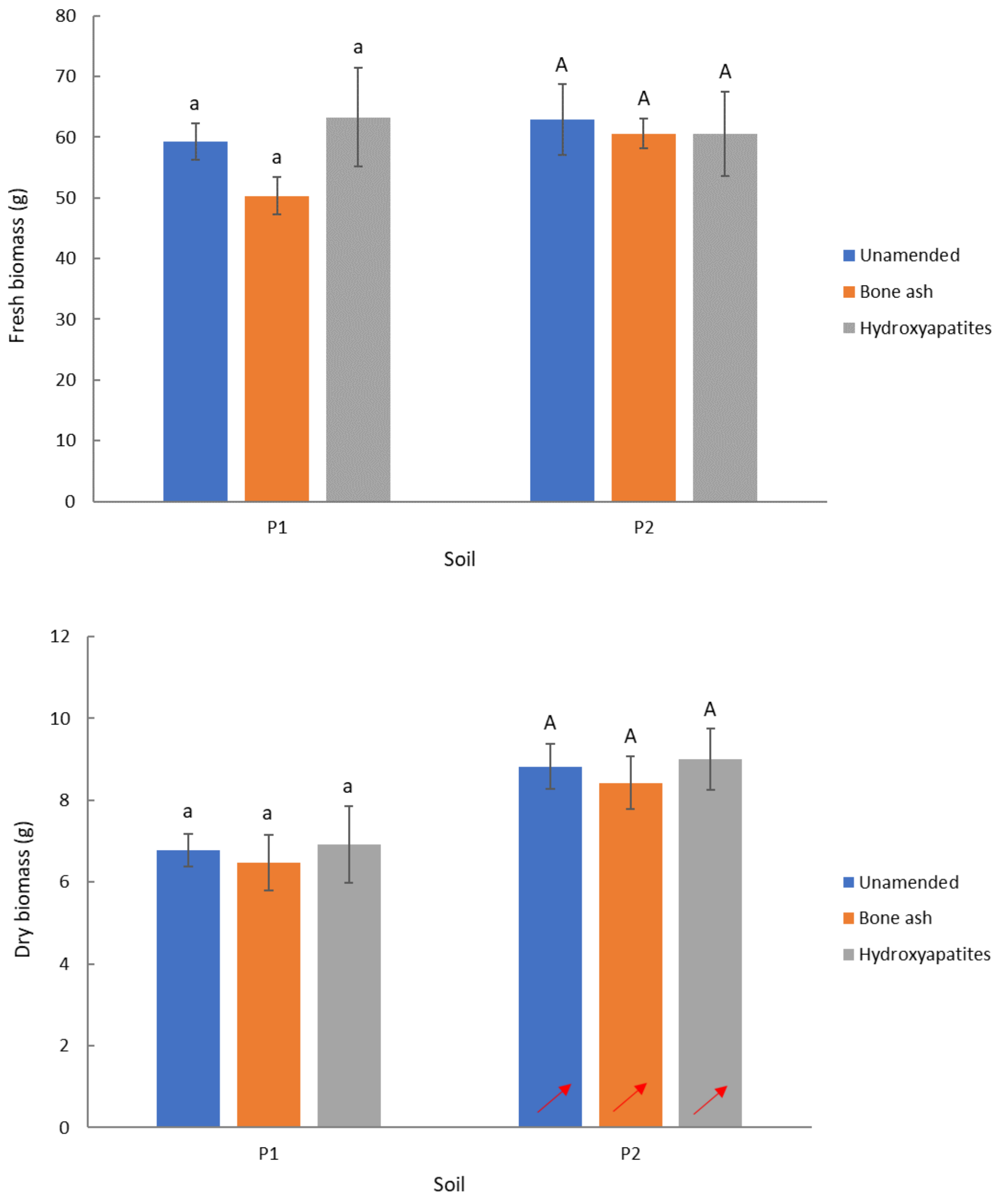
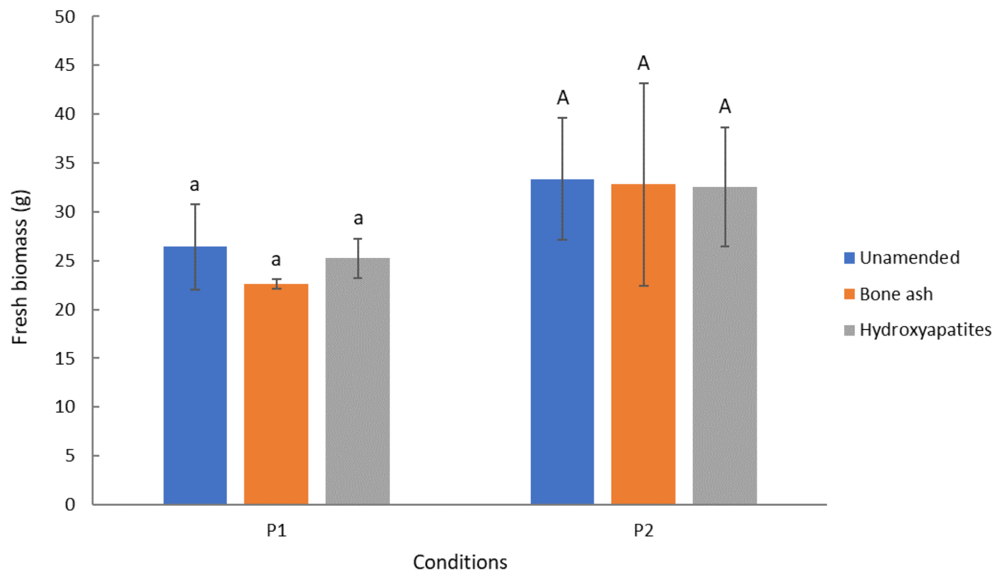


Figure 3: Fresh and dry biomass of ryegrass (g) at the first cut with the POMPEY soils



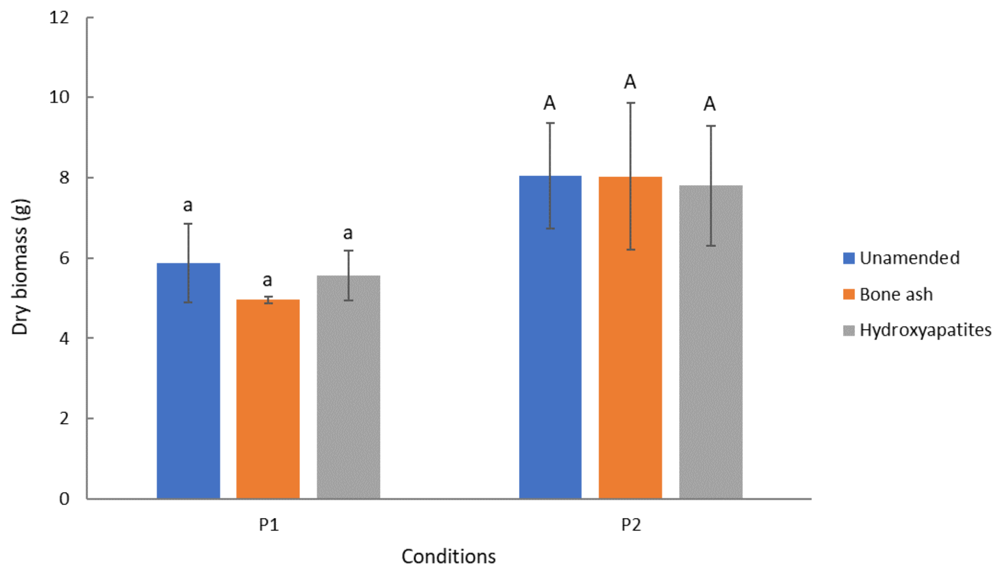


Figure 4: Fresh and dry biomass of ryegrass (g) at the second cut with the POMPEY soils

3.2.2 Metal concentrations

Figures 5, 6, 7 and **8** present the concentrations of Zn, Cu, Pb and Cu in ryegrass obtained at the first and second cut for all conditions with the soils from POMPEY.

Zn concentrations in ryegrass for P1 ranged between 132 and 172 mg kg^{-1} DW at the first cut and between 110 and 117 mg kg^{-1} DW at the second cut. Zn concentrations in ryegrass for P2 ranged between 357 and 387 mg kg^{-1} DW at the first cut and between 392 and 442 mg kg^{-1} DW at the second cut. No clear effect of the amendments and cuts were observed in P1 and P2.

Cu concentrations in ryegrass for P1 ranged between 10.3 and 11.4 mg kg^{-1} DW at the first cut and between 7.8 and 9.5 mg kg^{-1} DW at the second cut. Cu concentrations in ryegrass for P2 ranged between 11.6 and 11.9 mg kg^{-1} DW at the first cut and between 9.7 and 10.7 mg kg^{-1} DW at the second cut. No clear effect of the amendments was observed in P1 and P2. However, Cu concentrations in ryegrass tend to decrease at the second cut for both soils.

Pb concentrations in ryegrass for P1 ranged between 4.0 and 6.5 mg kg^{-1} DW at the first cut and between 4.3 and 6.1 mg kg^{-1} DW at the second cut. Pb concentrations in ryegrass for P2 ranged between 2.9 and 4.3 mg kg^{-1} DW at the first cut and between 4.6 and 4.7 mg kg^{-1} DW at the second cut. No effect of the amendments was observed in P1 and P2 and similar concentrations were obtained for both soils.

From a general point of view, metal concentrations in ryegrass were quite low for all the conditions, even if Zn concentrations in ryegrass are higher in P2 compared to P1. Different trends are observed concerning the effect of the cut on metal concentrations: i) no differences between the cuts for Zn and Pb, ii) an increase of Cd concentrations in ryegrass at the second cut, and iii) a slight decrease of Pb in ryegrass at the second cut.

Finally, the soil amendment by bone ash or hydroxyapatites has no effect on metal concentrations in ryegrass.

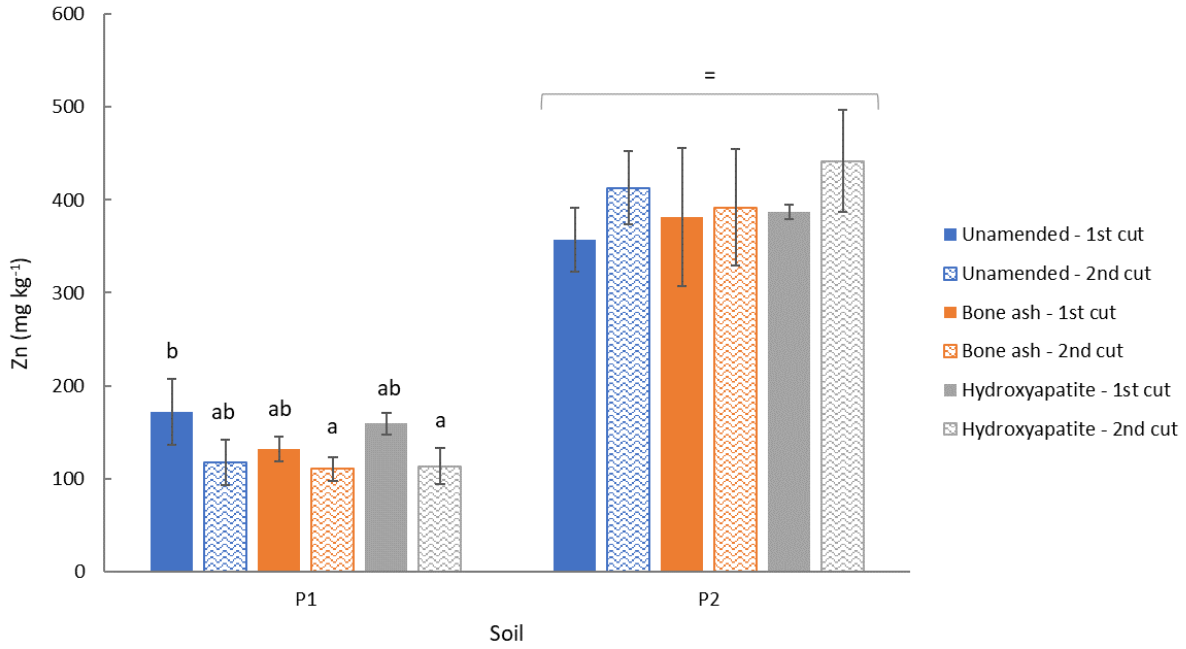


Figure 5: Zinc concentrations (mg kg^{-1}) in ryegrass at the first and second cut with the POMPEY soils

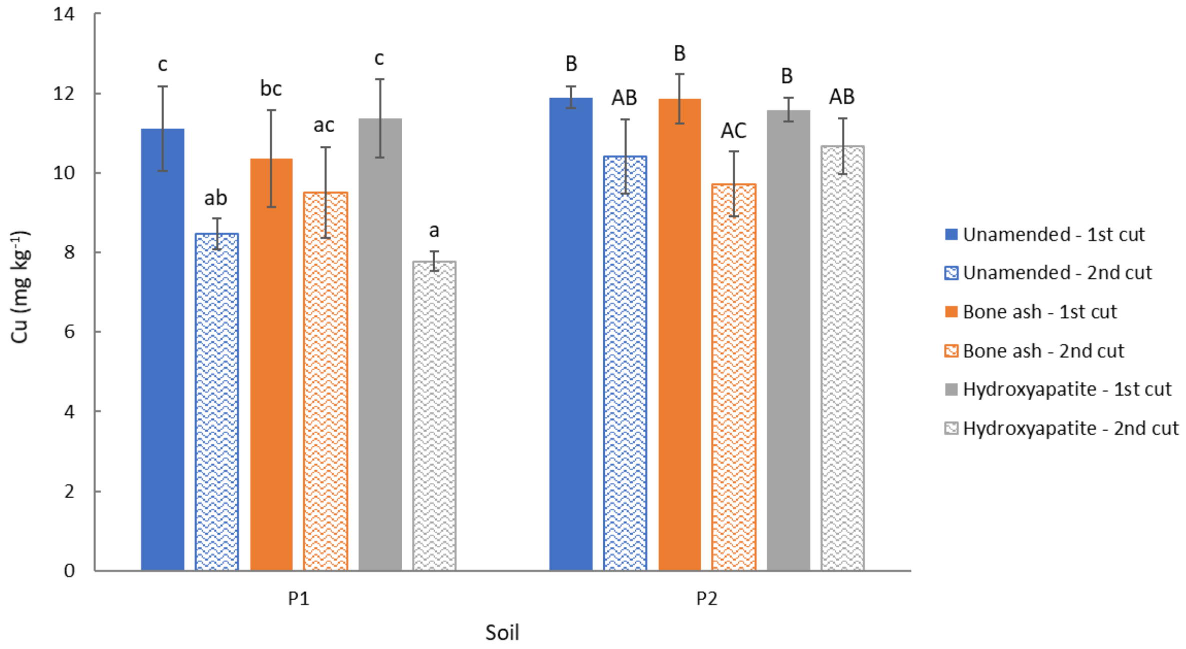


Figure 6: Copper concentrations (mg kg^{-1}) in ryegrass at the first and second cut with the POMPEY soils

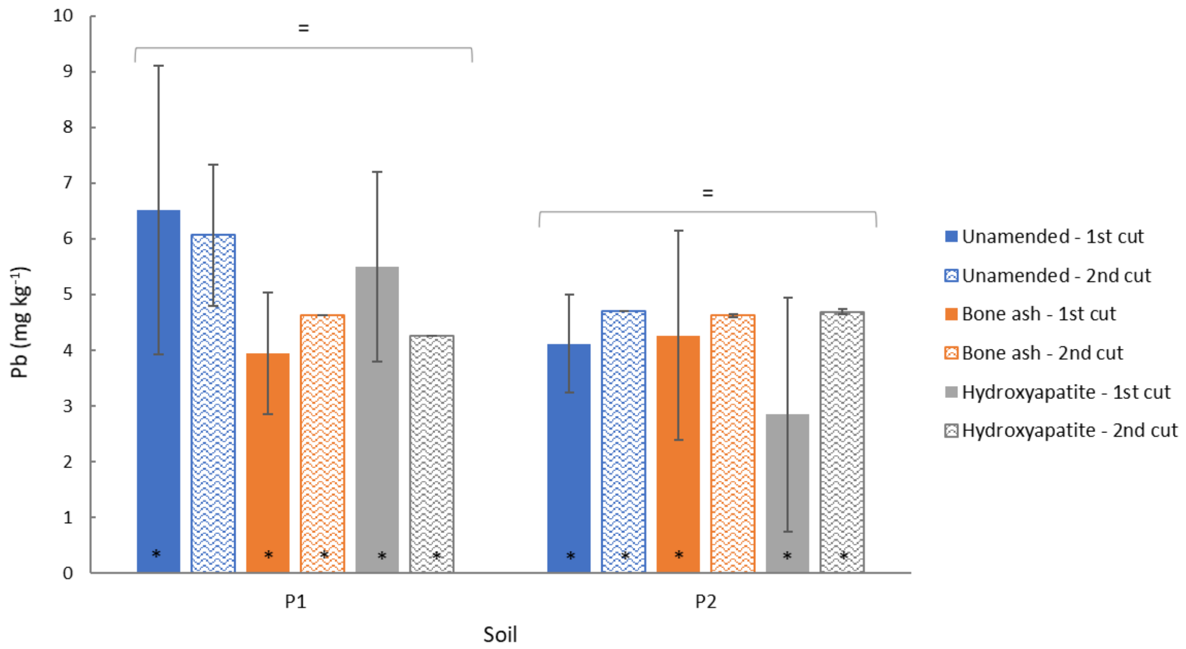


Figure 7: Lead concentrations (mg kg^{-1}) in ryegrass at the first and second cut with the POMPEY soils (: one or more samples are under the detection limit)*

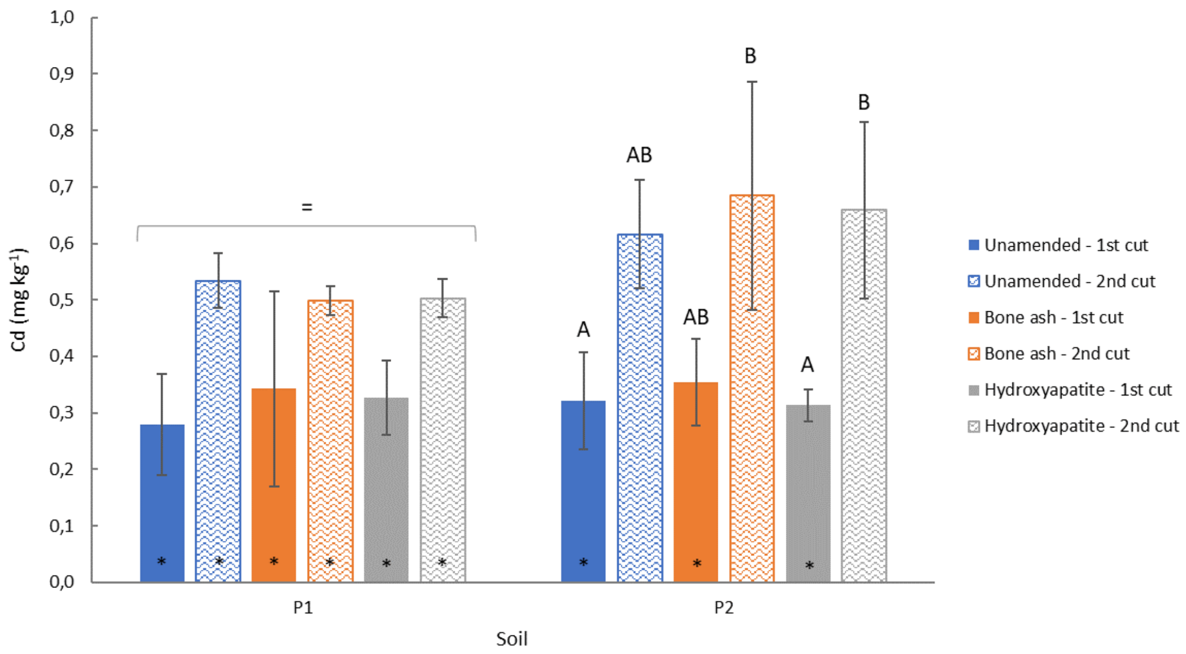


Figure 8: Cadmium concentrations (mg kg^{-1}) in ryegrass at the first and second cut with the POMPEY soils (: one or more samples are under the detection limit)*

3.2.3 Other elements

Due to the quite low concentration of Zn in ryegrass, we decided to determine the concentrations of Fe, Ca, Mg, Na and K in ryegrass. Indeed, these elements can also be considered as interesting for ecocatalyst production. **Table 5** presents the concentrations of these elements in ryegrass obtained at the first and second cut.

Table 5: Concentrations of Fe, K, Na, Ca, Mg (mg kg^{-1}) in ryegrass at the first and second cut with the POMPEY soils

Conditions	Amendment	Fe		K		Na		Ca		Mg	
		Mean (± *standard error*) - mg kg^{-1}									
		1^{st} cut	2^{nd} cut	1^{st} cut	2^{nd} cut	1^{st} cut	2^{nd} cut	1^{st} cut	2^{nd} cut	1^{st} cut	2^{nd} cut
P1	Unamended	22,4 *6,7*	32,1 *9,0*	24 768 *2 257*	15 048 *890*	568 *42*	3 506 *504*	8 035 *638*	8 965 *645*	2 089 *36*	2 616 *203*
	Bon ash	15,7 *3,6*	59,0 *27,9*	22 440 *1 230*	15 112 *1 775*	877 *36*	4 616 *571*	9 206 *938*	9 847 *393*	2 286 *178*	2 633 *121*
	Hydroxyapatites	20,5 *0,2*	39,9 *11,0*	30 535 *1 978*	15 924 *2 239*	541 *61*	3 786 *579*	8 424 *821*	8 518 *575*	2 268 *318*	2 324 *10*
P2	Unamended	20,4 *1,1*	38,0 *18,2*	38 190 *1 235*	22 555 *1 015*	275 *31*	2 651 *45*	10 049 *198*	10 361 *1 193*	3 212 *85*	3 875 *129*
	Bon ash	20,1 *3,7*	28,2 *4,2*	36 317 *5 206*	25 297 *2 404*	516 *118*	2 745 *251*	11 903 *1 375*	10 087 *665*	3 824 *589*	3 607 *262*
	Hydroxyapatites	19,7 *2,1*	33,1 *5,1*	36 477 *615*	25 191 *2 153*	377 *8*	2 832 *373*	10 622 *492*	10 774 *1 425*	3 779 *227*	3 806 *436*

- Fe

Fe concentrations in ryegrass were ranged between 15,7 and 22,4 mg kg^{-1} at the first cut for both soils and between 28,2 and 59,0 mg kg^{-1} at the second cut. Similar concentrations are obtained for both soils with no statistical effect of the amendments, except with bone ash for the soil P1 which increase Fe concentration in ryegrass. Moreover, Fe concentrations in ryegrass tend to increase at the second cut compared to the first one for both soils.

- K

For the soil P1, K concentrations in ryegrass were ranged between 22,4 and 30,5 g kg^{-1} at the first cut and between 15,0 and 15,9 g kg^{-1} at the second cut. For the soil P2, K concentrations in ryegrass were ranged between 36,3 and 38,2 g kg^{-1} at the first cut and between 22,6 and 25,3 g kg^{-1} at the second cut. K concentrations in soils were quite similar between the different soil treatments. However, K concentrations in ryegrass were significantly lower at the second cut compared to the first one. Finally, K concentrations were higher in ryegrass cultivated on the soil P2 compared to the soil P1.

- Na

For the soil P1, Na concentrations in ryegrass were ranged between 541 and 877 mg kg^{-1} at the first cut and between 3 506 and 4 616 mg kg^{-1} at the second cut. For the soil P2, Na concentrations in ryegrass were ranged between 275 and 516 mg kg^{-1} at the first cut and between 2 651 and 2 832 mg kg^{-1} at the second cut. The results highlight an increase of Na concentration in plants at the second cut compared to the first one. To finish, the amendments have no effect on Na concentrations.

- Ca

Ca concentrations in ryegrass were ranged between 8,0 and 9,8 g kg^{-1} for both cuts in P1 and between 10,0 and 11,9 mg kg^{-1} DW in P2, revealing small differences between the first and second cut. No difference was observed between the soil amendments. A slightly higher Ca concentrations in ryegrass cultivated on the soil P2 was observed.

- Mg

Mg concentrations in ryegrass were ranged between 2,1 and 2,6 g kg^{-1} for both cuts in P1 and between 3,2 and 3,9 mg kg^{-1} DW in P2, revealing small differences between the first and second cut. No difference was observed between the soil amendments. A slightly higher Mg concentrations in ryegrass cultivated on the soil P2 was observed.

To conclude, we observed small differences between the soils P1 and P2, even if some elements are slightly higher in ryegrass cultivated on the soil P2. The input of amendments on soils has negligible effects on element concentrations in ryegrass.

Thus, it has been decided to evaluate the efficiency of ryegrass, without distinction between the amendments, i) produced on P1 and ii) produced on P2.

3.3 Ecocatalyst production

Ecocatalyst will be produced with ryegrass cultivated on soils P1 and P2. Analyses and experiment are under progress.

4 CONCLUSION

Greenhouse experiments have been realized with soils samples collected from POMPEY in France. Two soils from the site have been used in this experiment. Moreover, we also evaluated the effects of two amendments: bone ash and hydroxyapatites.

The experiment with POMPEY soils revealed a very high ryegrass development but no effect of the amendments on soil properties. The Zn concentration in ryegrass was higher than those measured in ryegrass obtained with another experimental site. Based on these results, a Zn-enriched ecocatalyst will be produced and their efficiency in green chemistry will be evaluated.

To conclude, this greenhouse experiment revealed the interest of past metallurgic sites to cultivate and produce ryegrass rich in compounds of interests usable in green chemistry.