

Feed supplementation with molybdenum complexes improves honey bee health

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Supporting Information

Part I

Molybdenum in honey bees

Molybdenum in honey bees

Samples of honey bee workers were collected in different areas and environments to assess natural levels of molybdenum in France (see map, Figure SI-1), in Nea Moudania, Thessaloniki and Polygyros region of Greece (respectively urban/, industrial and natural environments) and in Moldova (forest environments in the region of Chisinau).

Bees are collected in accordance with [standard method X43-909](#) on “active environmental biomonitoring using honey bees.” The bees collected are foraging bees returning from their flight. These are the oldest bees in the colony and therefore those that have been exposed to external contaminants/metals for the longest period of time.

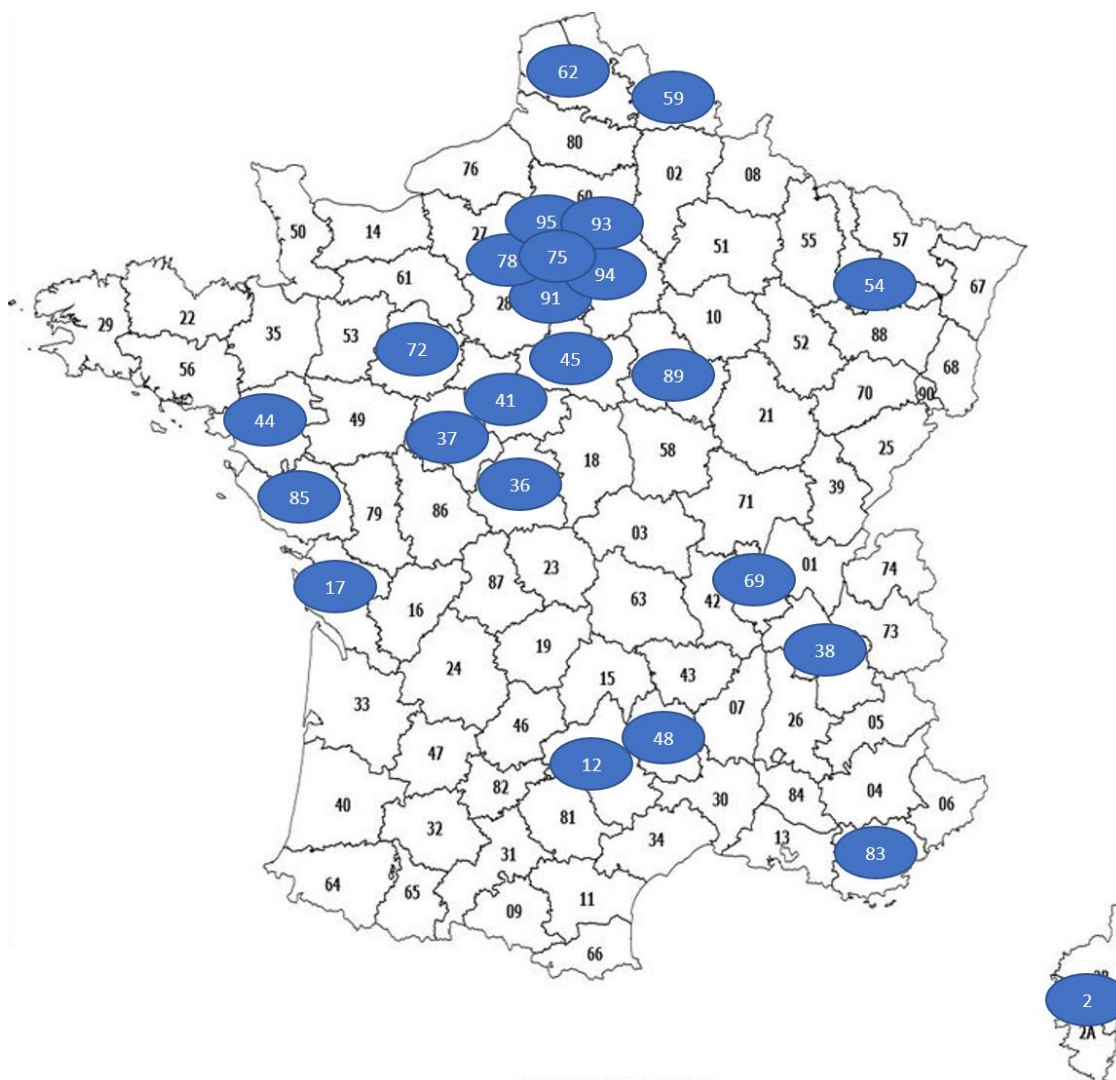


Figure SI-1. Map of the areas in France, where the bees were collected. Numbers correspond to the administrative numbers given to each department in France. The samples collected cover different type of environments and climates.

According to the [standard method X43-909](#), bee samples (30-50 bees/sample) correspond to a group of foraging bees taken from at least three hives in the same apiary in order to obtain a representative sample of the target apiary. The bee samples were frozen at -20°C after collection.

The bee samples were then dehydrated, mineralized and analyzed as follows :

Dehydration : collected honeybee (*Apis mellifera*) specimens were subjected to desiccation to remove moisture content and stabilize the samples for mineral analysis. Bees were first rinsed with deionized water to eliminate surface contaminants and then placed in clean, inert drying containers. Dessiccation was performed in a ventilated drying oven at a controlled temperature of 60 ± 5 °C for 24 to 48 hours, or until constant weight was achieved, indicating complete dehydration. The dried specimens were then cooled in a desiccator containing silica gel to prevent moisture reabsorption prior to mineralization and then ground in a mortar.

Mineralization : Mineralization is carried out in a closed microwave system (CEM MARS-6) with an iPREP reactor. The protocol consists of dissolving 0.25 g of sample in 8 mL of 70% nitric acid (JT Baker instra analyzed) - heating to 200°C for 20 minutes, then stabilizing at 200°C for 20 minutes and cooling for 20 minutes. The extract collected is perfectly clear and diluted to 50 mL with ultra-pure water (Merck - MillQ).

Analysis by ICP-MS : The standard method EN 13805 was then used to determine the molybdenum content by inductively coupled plasma mass spectrometry (ICP-MS).

The analyses of the molybdenum content by ICP-MS were performed by LEAV (Laboratoire de l'Environnement et d'Analyses de Vendée), La Roche-sur-Yon, France. The results found for Mo contents in ppm in dehydrated bee samples are gathered in Table SI-1. The uncertainty is estimated to ± 0.02 ppm ($\mu\text{g/g}$).

Table SI-1. Mo content in ppm ($\mu\text{g/g}$ of dried bee) as a function of sampling areas, month and year of sampling and environment. NAT, AGRI, URB, INDUS are given for Natural, Agricultural, Urban or Industrial environments, respectively.

N°	Sampling Area	Month	Year	Environment ^a	Mo content (ppm)
1	France (12)	4	2021	AGRI	0.31
2	France (12)	7	2021	AGRI	0.34
3	France (12)	9	2021	AGRI	0.36
4	France (17)	4	2013	AGRI	0.39
5	France (17)	4	2014	AGRI	1.19
6	France (17)	7	2014	AGRI	0.25
7	France (17)	9	2014	AGRI	0.22
8	France (17)	5	2015	AGRI	0.25
9	France (17)	7	2015	AGRI	0.34
10	France (17)	9	2015	AGRI	0.29
11	France (17)	5	2022	AGRI	0.37
12	France (17)	5	2022	AGRI	0.36

13	France (17)	5	2022	AGRI	0.49
14	France (17)	5	2022	AGRI	0.30
15	France (17)	5	2022	AGRI	0.41
16	France (17)	5	2022	AGRI	0.30
17	France (17)	5	2022	AGRI	0.35
18	France (17)	5	2022	AGRI	0.38
19	France (17)	5	2022	AGRI	0.27
20	France (17)	5	2022	AGRI	0.28
21	France (17)	5	2022	AGRI	0.42
22	France (17)	5	2022	AGRI	0.75
23	France (17)	5	2022	AGRI	0.26
24	France (17)	5	2022	AGRI	0.30
25	France (17)	5	2022	AGRI	0.42
26	France (17)	5	2022	AGRI	0.24
27	France (17)	6	2022	AGRI	0.33
28	France (17)	6	2022	AGRI	0.29
29	France (17)	6	2022	AGRI	0.39
30	France (17)	6	2022	AGRI	0.34
31	France (17)	6	2022	AGRI	0.23
32	France (17)	6	2022	AGRI	0.28
33	France (17)	6	2022	AGRI	0.34
34	France (17)	6	2022	AGRI	0.27
35	France (17)	7	2022	AGRI	0.22
36	France (17)	7	2022	AGRI	0.26
37	France (17)	7	2022	AGRI	0.36
38	France (17)	7	2022	AGRI	0.30
39	France (17)	7	2022	AGRI	0.23
40	France (17)	7	2022	AGRI	0.21
41	France (17)	7	2022	AGRI	0.16
42	France (17)	7	2022	AGRI	0.18
43	France (2)	4	2013	AGRI	1.35
44	France (2)	4	2013	AGRI	0.68
45	France (2)	6	2013	AGRI	0.43
46	France (2)	6	2013	AGRI	0.46
47	France (36)	4	2019	AGRI	0.09
48	France (36)	7	2019	AGRI	0.19
49	France (36)	9	2019	AGRI	0.21
50	France (36)	4	2020	AGRI	0.33
51	France (36)	7	2020	AGRI	0.35
52	France (36)	9	2020	AGRI	0.36
53	France (36)	4	2021	AGRI	0.4
54	France (36)	6	2021	AGRI	0.59
55	France (36)	9	2021	AGRI	0.48
56	France (37)	7	2014	NAT	0.31
57	France (37)	9	2014	NAT	0.47
58	France (37)	4	2015	NAT	0.21

59	France (37)	7	2015	NAT	0.16
60	France (37)	9	2015	NAT	0.20
61	France (37)	3	2016	NAT	0.17
62	France (37)	7	2016	NAT	0.23
63	France (37)	9	2016	NAT	0.29
64	France (37)	3	2017	NAT	0.41
65	France (37)	7	2017	NAT	0.21
66	France (37)	9	2017	NAT	0.21
67	France (37)	4	2018	NAT	0.33
68	France (37)	7	2018	NAT	0.298
69	France (37)	9	2018	NAT	0.234
70	France (37)	4	2019	NAT	0.08
71	France (37)	7	2019	NAT	0.21
72	France (37)	9	2019	NAT	0.59
73	France (37)	4	2020	NAT	0.025
74	France (37)	7	2020	NAT	0.15
75	France (37)	9	2020	NAT	0.36
76	France (37)	4	2021	NAT	0.2
77	France (37)	6	2021	NAT	0.35
78	France (37)	9	2021	NAT	0.38
79	France (38)	8	2016	URB	0.82
80	France (38)	8	2016	AGRI	0.44
81	France (38)	9	2016	URB	0.435
82	France (38)	9	2016	AGRI	0.34
83	France (41)	6	2013	AGRI	0.45
84	France (41)	4	2014	AGRI	1.31
85	France (41)	7	2014	AGRI	0.39
86	France (41)	9	2014	AGRI	0.37
87	France (41)	4	2015	AGRI	0.23
88	France (41)	7	2015	AGRI	0.19
89	France (41)	9	2015	AGRI	0.24
90	France (41)	3	2016	AGRI	0.28
91	France (41)	7	2016	AGRI	0.37
92	France (41)	9	2016	AGRI	0.33
93	France (41)	3	2017	AGRI	0.38
94	France (41)	7	2017	AGRI	0.28
95	France (41)	9	2017	AGRI	0.54
96	France (41)	4	2018	AGRI	0.29
97	France (41)	7	2018	AGRI	0.45
98	France (41)	9	2018	AGRI	0.40
99	France (41)	4	2019	AGRI	0.08
100	France (41)	7	2019	AGRI	0.23
101	France (41)	9	2019	AGRI	0.24
102	France (41)	4	2020	AGRI	0.025
103	France (41)	7	2020	AGRI	0.35
104	France (41)	9	2020	AGRI	0.34

105	France (41)	4	2021	AGRI	0.20
106	France (41)	6	2021	AGRI	0.31
107	France (41)	9	2021	AGRI	0.33
108	France (44)	6	2014	AGRI	0.35
109	France (44)	7	2014	AGRI	0.41
110	France (44)	10	2014	AGRI	0.22
111	France (44)	4	2015	AGRI	0.3
112	France (44)	7	2015	AGRI	0.34
113	France (44)	9	2015	AGRI	0.34
114	France (44)	4	2016	AGRI	0.35
115	France (44)	7	2016	AGRI	0.25
116	France (44)	9	2016	AGRI	0.395
117	France (45)	4	2013	AGRI	0.34
118	France (45)	6	2013	AGRI	0.28
119	France (45)	4	2014	AGRI	1.19
120	France (45)	7	2014	AGRI	0.48
121	France (45)	9	2014	AGRI	0.33
122	France (45)	5	2015	AGRI	0.18
123	France (45)	7	2015	AGRI	0.26
124	France (45)	9	2015	AGRI	0.41
125	France (45)	3	2016	AGRI	0.36
126	France (45)	7	2016	AGRI	0.48
127	France (45)	9	2016	AGRI	0.535
128	France (45)	3	2017	AGRI	0.44
129	France (45)	7	2017	AGRI	0.41
130	France (45)	9	2017	AGRI	0.47
131	France (45)	4	2018	AGRI	0.58
132	France (45)	7	2018	AGRI	0.62
133	France (45)	9	2018	AGRI	0.395
134	France (45)	4	2019	AGRI	0.19
135	France (45)	7	2019	AGRI	0.42
136	France (45)	9	2019	AGRI	0.44
137	France (45)	4	2020	AGRI	0.025
138	France (45)	7	2020	AGRI	0.28
139	France (45)	9	2020	AGRI	0.49
140	France (45)	4	2021	AGRI	0.35
141	France (45)	6	2021	AGRI	0.69
142	France (45)	9	2021	AGRI	0.36
143	France (48)	4	2021	NAT	0.45
144	France (48)	7	2021	NAT	0.38
145	France (48)	9	2021	NAT	0.35
146	France (54)	6	2019	AGRI	0.35
147	France (54)	6	2019	AGRI	0.29
148	France (54)	7	2019	AGRI	0.35
149	France (54)	7	2019	AGRI	0.21
150	France (54)	9	2019	AGRI	0.22

151	France (54)	9	2019	AGRI	0.24
152	France (54)	6	2020	AGRI	0.30
153	France (54)	6	2020	AGRI	0.28
154	France (54)	8	2020	AGRI	0.31
155	France (54)	8	2020	AGRI	0.24
156	France (54)	9	2020	AGRI	0.28
157	France (54)	9	2020	AGRI	0.29
158	France (59)	9	2020	AGRI	0.52
159	France (59)	9	2020	AGRI	0.57
160	France (59)	9	2020	AGRI	0.63
161	France (59)	6	2021	AGRI	0.43
162	France (59)	6	2021	AGRI	0.42
163	France (59)	6	2021	AGRI	0.41
164	France (59)	6	2021	AGRI	0.44
165	France (59)	8	2021	AGRI	0.39
166	France (59)	8	2021	AGRI	0.52
167	France (59)	8	2021	AGRI	0.47
168	France (59)	8	2021	AGRI	0.46
169	France (62)	5	2016	AGRI	0.64
170	France (62)	7	2016	AGRI	0.57
171	France (62)	9	2016	AGRI	0.37
172	France (62)	5	2017	AGRI	0.71
173	France (62)	7	2017	AGRI	1.48
174	France (62)	9	2017	AGRI	0.78
175	France (69)	9	2021	URB	0.42
176	France (72)	7	2017	NAT	0.41
177	France (72)	7	2017	NAT	0.24
178	France (72)	9	2017	NAT	0.43
179	France (72)	9	2017	NAT	0.27
180	France (72)	5	2018	NAT	0.43
181	France (72)	5	2018	NAT	0.395
182	France (75)	9	2015	URB	0.68
183	France (75)	4	2019	URB	0.27
184	France (75)	4	2019	URB	0.30
185	France (75)	7	2019	NAT	2.31
186	France (75)	7	2019	NAT	0.69
187	France (75)	9	2019	AGRI	0.49
188	France (75)	9	2019	AGRI	0.51
189	France (75)	4	2020	URB	0.36
190	France (75)	7	2020	NAT	0.64
191	France (75)	9	2020	AGRI	0.77
192	France (78)	4	2013	URB	0.35
193	France (78)	6	2013	URB	0.47
194	France (78)	4	2014	URB	1.12
195	France (78)	7	2014	URB	0.46
196	France (78)	9	2014	URB	0.39

197	France (78)	4	2015	URB	0.26
198	France (78)	7	2015	URB	0.49
199	France (78)	9	2015	URB	0.40
200	France (83)	4	2013	NAT	0.45
201	France (83)	4	2013	NAT	0.42
202	France (83)	4	2013	NAT	0.35
203	France (83)	4	2014	NAT	0.53
204	France (83)	7	2014	NAT	0.36
205	France (83)	9	2014	NAT	0.35
206	France (85)	3	2022	NAT	0.23
207	France (85)	4	2022	NAT	0.11
208	France (85)	5	2022	NAT	0.10
209	France (85)	5	2022	NAT	0.09
210	France (85)	6	2022	NAT	0.07
211	France (85)	6	2022	NAT	0.23
212	France (85)	6	2022	NAT	0.14
213	France (85)	6	2022	AGRI	0.23
214	France (85)	6	2022	AGRI	0.14
215	France (85)	7	2022	NAT	0.31
216	France (85)	7	2022	NAT	0.21
217	France (85)	7	2022	AGRI	0.31
218	France (85)	7	2022	AGRI	0.25
219	France (85)	8	2022	NAT	0.25
220	France (85)	8	2022	AGRI	0.25
221	France (85)	9	2022	NAT	0.27
222	France (85)	9	2022	AGRI	0.27
223	France (89)	4	2020	URB	0.37
224	France (89)	4	2020	AGRI	0.41
225	France (89)	4	2021	URB	0.39
226	France (89)	4	2021	AGRI	0.26
227	France (89)	6	2021	URB	0.72
228	France (89)	6	2021	AGRI	0.46
229	France (89)	9	2021	URB	0.59
230	France (89)	9	2021	AGRI	0.48
231	France (91)	6	2013	AGRI	0.23
232	France (91)	5	2015	AGRI	0.2
233	France (91)	7	2015	AGRI	0.19
234	France (91)	9	2015	AGRI	0.27
235	France (91)	4	2019	AGRI	0.13
236	France (91)	7	2019	AGRI	0.35
237	France (91)	9	2019	AGRI	0.42
238	France (91)	4	2020	AGRI	0.025
239	France (91)	7	2020	AGRI	0.32
240	France (91)	9	2020	AGRI	0.31
241	France (91)	4	2021	AGRI	0.21
242	France (91)	6	2021	AGRI	0.29

243	France (91)	9	2021	AGRI	0.38
244	France (91)	3	2022	AGRI	0.82
245	France (91)	4	2022	AGRI	0.49
246	France (91)	5	2022	AGRI	0.45
247	France (91)	5	2022	AGRI	0.46
248	France (91)	6	2022	AGRI	0.50
249	France (91)	6	2022	AGRI	0.39
250	France (91)	7	2022	AGRI	0.35
251	France (91)	7	2022	AGRI	0.41
252	France (91)	8	2022	AGRI	0.40
253	France (91)	8	2022	AGRI	0.42
254	France (91)	9	2022	AGRI	0.44
255	France (91)	9	2022	AGRI	0.49
256	France (91)	10	2022	AGRI	0.48
257	France (93)	4	2019	URB	0.28
258	France (93)	7	2019	NAT	0.55
259	France (93)	9	2019	AGRI	0.25
260	France (94)	4	2019	URB	0.40
261	France (94)	7	2019	NAT	0.67
262	France (94)	9	2019	AGRI	0.66
263	France (94)	4	2020	URB	0.46
264	France (94)	7	2020	NAT	0.54
265	France (94)	9	2020	AGRI	0.68
266	France (95)	4	2015	URB	0.35
267	France (95)	4	2015	NAT	0.37
268	France (95)	7	2015	URB	0.46
269	France (95)	7	2015	NAT	0.27
270	France (95)	9	2015	URB	0.51
271	France (95)	9	2015	NAT	0.26
272	France (95)	3	2016	URB	0.35
273	France (95)	7	2016	URB	0.40
274	France (95)	9	2016	URB	0.385
275	Greece	4	2022	URB	0.48
276	Greece	4	2022	URB	0.32
277	Greece	4	2022	INDUS	0.34
278	Greece	4	2022	INDUS	0.52
279	Greece	4	2022	NAT	0.21
280	Greece	4	2022	NAT	0.22
281	Greece	5	2022	URB	0.61
282	Greece	5	2022	URB	0.56
283	Greece	5	2022	INDUS	0.53
284	Greece	5	2022	INDUS	0.46
285	Greece	5	2022	NAT	0.32
286	Greece	5	2022	NAT	0.26
287	Greece	6	2022	INDUS	0.23
288	Greece	6	2022	INDUS	0.38

289	Greece	6	2022	URB	0.53
290	Greece	6	2022	URB	0.34
291	Greece	6	2022	NAT	0.23
292	Greece	6	2022	NAT	0.38
293	Greece	7	2022	INDUS	0.28
294	Greece	7	2022	INDUS	0.29
295	Greece	7	2022	URB	0.50
296	Greece	7	2022	URB	0.47
297	Greece	7	2022	NAT	0.28
298	Greece	7	2022	NAT	0.29
299	Greece	8	2022	INDUS	0.32
300	Greece	8	2022	INDUS	0.32
301	Greece	8	2022	URB	0.38
302	Greece	8	2022	URB	0.35
303	Greece	8	2022	NAT	0.32
304	Greece	8	2022	NAT	0.32
305	Greece	9	2022	INDUS	0.43
306	Greece	9	2022	INDUS	0.25
307	Greece	9	2022	URB	0.53
308	Greece	9	2022	URB	0.55
309	Greece	9	2022	NAT	0.43
310	Greece	9	2022	NAT	0.25
311	Moldova	3	2022	NAT	0.50
312	Moldova	4	2022	NAT	0.67
313	Moldova	5	2022	NAT	1.20
314	Moldova	6	2022	NAT	0.93
315	Moldova	7	2022	NAT	0.53
316	Moldova	8	2022	NAT	0.30
317	Moldova	9	2022	NAT	0.26
318	Moldova	10	2022	NAT	0.30

The analysis of the 318 samples shown in the table SI-1 gives an overall **average value of 0.39 ppm for Mo in honey bees**. The distribution of Mo-content within these 318 bee samples are represented in the Figure SI.2. 75% of the bees analyzed display a Mo content in the 0.2-0.5 ppm range.

As shown in Table SI.2, this average content varies according to the environment but the standard deviation is rather high, especially in natural environment. It remains around 0.39 ppm in agricultural environment, slightly decreases to 0.36 ppm in natural environment, but rises slightly to 0.44 ppm in urban or industrial environments, suggesting an anthropogenic contribution in this case.

Table SI.2. Average values found for Mo content in bees as a function of their environment.

	NAT	AGRI	URB	INDUS
Nb of samples	78	188	40	12
Mean Mo (ppm)	0.364	0.386	0.463	0.363
Standard Deviation SD	0.291	0.207	0.162	0.102

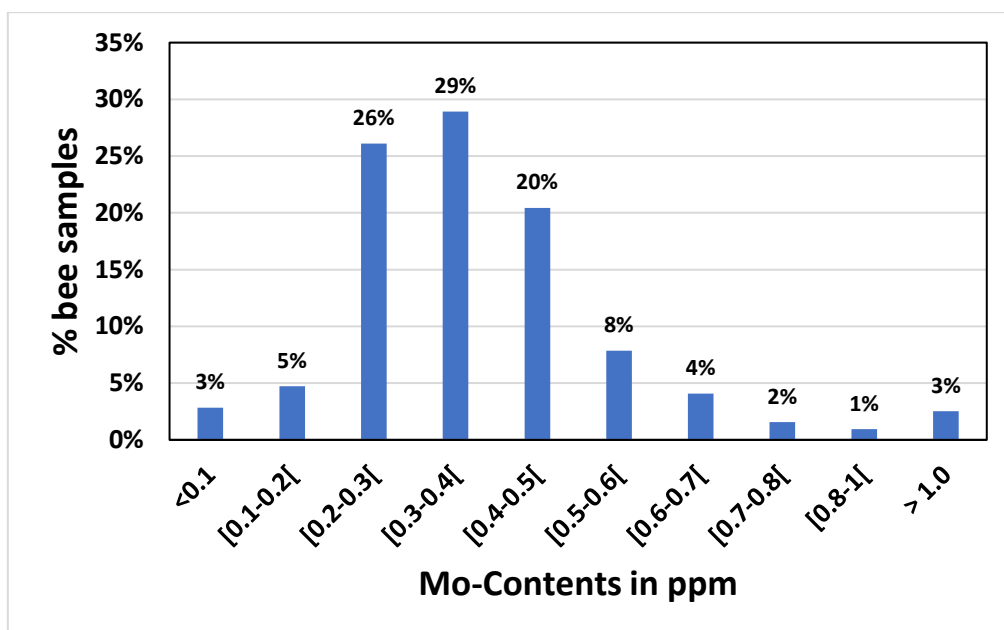


Figure SI-2. Representation of the distribution of Mo-content in the 318 bee samples analyzed. The main part, 75%, are found between 0.2 and 0.5 ppm.