

SUPPLEMENTARY MATERIAL FOR THE ARTICLE

“Sustainable Impact of Sewage Sludge Use on Soil Organic Matter and Nutrients: A Meta-Analysis”

Note about the statistical analysis

In sequence we show the code used for apply the statistical analysis in the meta-analysis (meta regression models) research on effects of sewage sludge application on soil attributes.

The basic workflow was (1) importing the Excel file dataset with the data (each line as an observation), then (2) calculating the effect size as the log transformed ratio of means, after which (3) applying a meta regression model. With the model done, the minimal data for run the meta regression analysis are:

Outcome variables	Organic matter, N, P, Zn, Cu
Moderators	sewage sludge concentration
	continent
	experimental condition
Study id	Name of studies or alphanumerical codes
Comparison	Numerical identifier of each observation within each study

Response variables		
Variable	Grouping	Description
n1i	Treatment group (with sewage sludge application)	Number of individuals in each comparison
m1i		Mean of the outcome variable
sd1i		Standard deviation of the mean
n2i	Control group	Number of individuals in each comparison
m2i		Mean of the outcome variable
sd2i		Standard deviation of the mean

Then, the function *escalc()* was applied to calculate the effect size, after which a meta regression model was performed with the *rma.mv()* function.

R packages used:

“ metafor ”	Viechtbauer, W. (2010). Conducting meta-analyses in R with the metafor package. <i>Journal of Statistical Software</i> , 36(3), 1-48. doi:10.18637/jss.v036.i03
“ tidyverse ”	Wickham H. et al. (2019). “Welcome to the tidyverse.” <i>Journal of Open Source Software</i> , 4(43), 1686. doi:10.21105/joss.01686.
“ ggthemes ”	Arnold J (2024). <i>_ggthemes: Extra Themes, Scales and Geoms for 'ggplot2'</i> . R package version 5.1.0, <https://CRAN.R-project.org/package=ggthemes> .
“ readxl ”	Wickham H, Bryan J (2023). <i>_readxl: Read Excel Files</i> . R package version 1.4.3,

	< https://CRAN.R-project.org/package=readxl >.
“writexl”	Ooms J (2024). _writexl: Export Data Frames to Excel 'xlsx' Format_. R package version 1.5.0, < https://CRAN.R-project.org/package=writexl >.
“gridExtra”	Auguie B (2017). _gridExtra: Miscellaneous Functions for "Grid" Graphics_. R package version 2.3, < https://CRAN.R-project.org/package=gridExtra >.
“emmeans”	Lenth R (2024). _emmeans: Estimated Marginal Means, aka Least-Squares Means_. R package version 1.10.2, < https://CRAN.R-project.org/package=emmeans >.

```
##Importing dataset

data <- read_xlsx("xlsx file path")

## calculating the effect sizes with the function escalc (metafor
package), using as the measure the log transformed ratio of means "ROM"

data <- escalc(measure = "ROM", n1i = n1i, n2i = n2i, m1i = m1i, m2i =
m2i, sd1i = sd1i, sd2i = sd2i, data = data)

## checking if there are not zero values in the vi column:

min(data$vi)
```

ORGANIC MATTER

Multivariate Meta-Analysis Model (k = 56; method: REML)

```
data_om <- data %>%
  + filter(outcome == "om")

model_om <- rma.mv(yi, vi, mods = ~ ss_rate + region + local, random = ~1
| study_n/comparison, data = data_om)

summary(model_om)
```

logLik	Deviance	AIC	BIC	AICc
-365.2941	730.5883	750.5883	769.3003	756.5342

Variance Components:

estim	sqrt	nlvls	fixed	factor
sigma^2.1	0.1672	0.4089	24	no
				study_n
sigma^2.2	0.0285	0.1689	35	no
				study_n/comparison

Test for Residual Heterogeneity:

QE(df = 48) = 2518.4932, p-val < .0001

Test of Moderators (coefficients 2:8):

QM(df = 7) = 427.6831, p-val < .0001

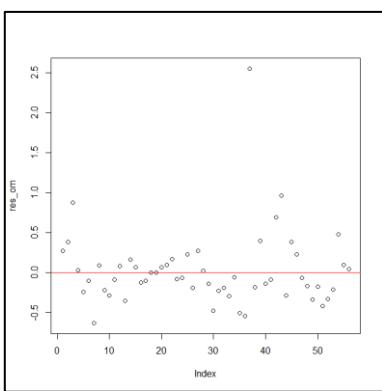
Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub	
intrcpt	0.7493	0.1861	4.0266	<.0001	0.3846	1.1140	***
ss_ratelow	-0.3709	0.0181	-20.4597	<.0001	-0.4065	-0.3354	***
ss_ratemedium	-0.1200	0.0143	-8.3891	<.0001	-0.1480	-0.0920	***
regionamerica	-0.1167	0.2387	-0.4888	0.6250	-0.5846	0.3512	
regionasia	0.0189	0.2471	0.0763	0.9392	-0.4655	0.5032	
regioneurope	0.3513	0.4800	0.7318	0.4643	-0.5895	1.2921	
regionoceania	0.0260	0.3675	0.0707	0.9437	-0.6943	0.7462	
locallab	0.3556	0.2274	1.5642	0.1178	-0.0900	0.8012	

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Generating the residuals of the model:

```
res_om <- resid(model_om)
plot(res_om)
abline(h = 0, col = "red", size = 1)
```



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Publication Bias (organic matter)

By applying the square root of the variance as a moderator in the model:

```

model_ombias <- rma.mv(yi, vi, mods = ~ sqrt(vi), random = ~1 |
study_n/comparison, data = data_om)

summary(model_ombias)

```

Multivariate Meta-Analysis Model (k = 56; method: REML)

logLik	Deviance	AIC	BIC	AICc
-572.3179	1144.6359	1152.6359	1160.5918	1153.4522

Variance Components:

estim	sqrt	nlvls	fixed	factor
sigma^2.1	0.1961	0.4428	24	no study_n
sigma^2.2	0.0120	0.1095	35	no study_n/comparison

Test for Residual Heterogeneity:

QE(df = 54) = 5457.0852, p-val < .0001

Test of Moderators (coefficient 2):

QM(df = 1) = 5.9210, p-val = 0.0150

Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub	
intrcpt	0.7203	0.1100	6.5450	<.0001	0.5046	0.9359	***
sqrt(vi)	-1.8843	0.7744	-2.4333	0.0150	-3.4020	-0.3666	*
					--		

Calculating the global effect of the treatment, proportional to moderators:

```

sav_om <- emmprep(model_om)

emmeans(sav_om, specs = "1", type = "response", weights = "proportional")

1   response  SE df asymp.LCL asymp.UCL
overall  1.83 0.169 Inf   1.52    2.19

```

Results are averaged over the levels of: ss_rate, region, local

Confidence level used: 0.95

Intervals are back-transformed from the log scale

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Calculating the predict values, transforming back from log to the effect size scale (*exp* function):

```
pred_om <- data.frame(predict(model_om, newmods = rbind(c(1,0,0,0,0,0,0),  
c(0,1,0,0,0,0,0), c(0,0,1,0,0,0,0), c(0,0,0,1,0,0,0), c(0,0,0,0,1,0,0),  
c(0,0,0,0,0,1,0), c(0,0,0,0,0,0,1)), transf = exp))
```

Exporting the predict values dataframe and after editing it, re-import to R environment.

```
write_xlsx(pred_om, path)
```

editing in excel file (inserting the lines [*italic*] of reference predict values [back transformed] in each moderator (“mod” column):

mod	level	pred	ci.lb	ci.ub
<i>rate</i>	<i>High</i>	2.2032	1.5343	3.1639
rate	Low	1.512365	1.052761	2.172619
rate	Medium	1.937844	1.34944	2.782812
<i>continent</i>	<i>Africa</i>	2.2032	1.5343	3.1639
continent	America	1.896823	1.377439	2.612051
continent	Asia	2.173237	1.535375	3.076096
continent	Europe	3.021868	1.276004	7.156474
continent	Oceania	2.182662	1.176556	4.049119
<i>condition</i>	<i>Field</i>	2.2032	1.5343	3.1639
condition	Greenhouse	3.108562	1.873381	5.158137

importing the predict data:

```
pred_om <- read_xlsx("xlsx path")  
  
pred_om_rate <- pred_om %>%  
+   filter(mod == "rate") %>%  
+   mutate(level = factor(level, levels = c("Low", "Medium", "High")))  
  
pred_om_continent <- pred_om %>%  
+   filter(mod == "continent") %>%  
+   mutate(level = factor(level, levels = c("Africa", "America", "Asia",  
"Europe", "Oceania")))  
  
pred_om_condition <- pred_om %>%  
+   filter(mod == "condition") %>%  
+   mutate(level = factor(level, levels = c("Field", "Greenhouse")))  
  
--
```

Drawing the graphs:

```

f1 <- ggplot(pred_om_rate, aes(x = pred, y = level)) +
  geom_point(position = position_dodge(width = 0.35), size = 3) +
  geom_errorbarh(aes(xmin = ci.lb, xmax = ci.ub), height = 0.1, position =
  position_dodge(width = 0.30), linewidth = 0.7) +
  theme_classic() +
  labs(y = "Sewage sludge rate",
       x = "Response Ratio") +
  theme(axis.text = element_text(size = 12, colour = "black")) +
  theme(legend.position = "none",
        axis.title = element_text(size = 12, colour = "black"),
        panel.border = element_blank()) +
  scale_x_continuous(limits = c(0,3.5), expand = c(0,0), breaks = seq(0,
  3, by = 1)) +
  geom_vline(xintercept = 1, linetype = "dashed", color = "#000000", size
  = 1)

f2 <- ggplot(pred_om_continent, aes(x = pred, y = level)) +
  geom_point(position = position_dodge(width = 0.35), size = 3) +
  geom_errorbarh(aes(xmin = ci.lb, xmax = ci.ub), height = 0.1, position =
  position_dodge(width = 0.30), linewidth = 0.7) +
  theme_classic() +
  labs(y = "Sewage sludge rate",
       x = "Response Ratio") +
  theme(axis.text = element_text(size = 12, colour = "black")) +
  theme(legend.position = "none",
        axis.title = element_text(size = 12, colour = "black"),
        panel.border = element_blank()) +
  scale_x_continuous(limits = c(0,8), expand = c(0,0), breaks = seq(0, 8,
  by = 1)) +
  geom_vline(xintercept = 1, linetype = "dashed", color = "#000000", size
  = 1)

```

```

f3 <- ggplot(pred_om_condition, aes(x = pred, y = level)) +
  geom_point(position = position_dodge(width = 0.35), size = 3) +
  geom_errorbarh(aes(xmin = ci.lb, xmax = ci.ub), height = 0.1, position =
  position_dodge(width = 0.30), linewidth = 0.7) +
  theme_classic() +
  labs(y = "Sewage sludge rate",
       x = "Response Ratio") +
  theme(axis.text = element_text(size = 12, colour = "black")) +
  theme(legend.position = "none",
        axis.title = element_text(size = 12, colour = "black"),
        panel.border = element_blank()) +
  scale_x_continuous(limits = c(0,6), expand = c(0,0), breaks = seq(0, 6,
  by = 1)) +
  geom_vline(xintercept = 1, linetype = "dashed", color = "#000000", size
  = 1)

```

The same code is repeated for all other variable outcomes (nitrogen, phosphorus, zinc and copper).

Following there are the outputs of the analysis.

NITROGEN

Multivariate Meta-Analysis Model (k = 51; method: REML)

logLik	Deviance	AIC	BIC	AICC
-36.7461	73.4923	93.4923	111.1043	100.3673

Variance Components:

estim	sqrt	nlvls	fixed	factor
sigma^2.1	0.0128	0.1131	19	no study
sigma^2.2	0.1155	0.3398	28	no study/comparison

Test for Residual Heterogeneity:

QE(df = 43) = 854.8001, p-val < .0001

Test of Moderators (coefficients 2:8):

QM(df = 7) = 339.2645, p-val < .0001

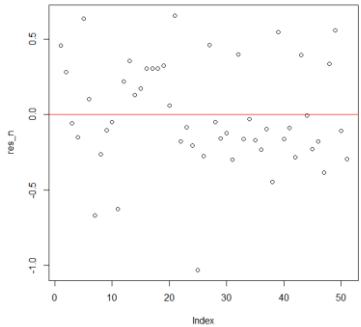
Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub	
intrcpt	0.6494	0.1162	5.5883	<.0001	0.4216	0.8772	***
ss_ratelow	-0.6523	0.0359	-18.1932	<.0001	-0.7226	-0.5821	***
ss_ratemedium	-0.2473	0.0277	-8.9318	<.0001	-0.3016	-0.1931	***
regionamerica	0.3416	0.1935	1.7654	0.0775	-0.0376	0.7209	.
regionasia	0.4569	0.1923	2.3761	0.0175	0.0800	0.8337	*
regioneurope	0.4659	0.2835	1.6431	0.1004	-0.0898	1.0215	
regionoceania	0.5922	0.2844	2.0824	0.0373	0.0348	1.1497	*
locallab	0.3074	0.1080	2.8470	0.0044	0.0958	0.5190	**

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

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Residuals:



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Publication Bias (nitrogen)

Multivariate Meta-Analysis Model ($k = 51$; method: REML)

logLik	Deviance	AIC	BIC	AICc
-207.5386	415.0772	423.0772	430.6445	423.9863

Variance Components:

estim	sqrt	nlvls	fixed	factor
sigma^2.1	0.0504	0.2246	19	no
study_n				
sigma^2.2	0.0488	0.2209	28	no
study_n/comparison				

Test for Residual Heterogeneity:

QE(df = 49) = 1419.8168, p-val < .0001

Test of Moderators (coefficient 2):

QM(df = 1) = 6.0045, p-val = 0.0143

Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub	
intrcpt	0.8266	0.1122	7.3676	<.0001	0.6067	1.0465	***
sqrt(vi)	-2.5547	1.0425	-2.4504	0.0143	-4.5980	-0.5113	*
Signif. codes:	0	***	0.001	**	0.01	*	.
	0.05	.'	0.1	'	1		

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Global effect of the treatment

1	response	SE	df	asymp.LCL	asymp.UCL
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overall **1.82** 0.134 Inf 1.58 2.1

Results are averaged over the levels of: ss_rate, region, local

Confidence level used: 0.95

Intervals are back-transformed from the log scale

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Predict values (already back transformed from log scale)

mod	level	pred	ci.lb	ci.ub
rate	High	1.9143	1.524399	2.404159
rate	Low	0.99709	0.788997	1.260065
rate	Medium	1.494949	1.188215	1.880866
continent	Africa	1.9143	1.524399	2.404159
continent	America	2.694051	1.983022	3.660026
continent	Asia	3.023128	2.220314	4.116222
continent	Europe	3.050373	1.834377	5.072446
continent	Oceania	3.461339	2.076953	5.768484
condition	Field	1.9143	1.524399	2.404159
condition	Greenhouse	2.603275	1.938831	3.495426

PHOSPHORUS

Multivariate Meta-Analysis Model (k = 61; method: REML)

logLik	Deviance	AIC	BIC	AICc
-501.6825	1003.3650	1023.3650	1043.0679	1028.6031

Variance Components:

estim	sqrt	nlvls	fixed	factor
sigma^2.1	0.6043	0.7773	27	no study
sigma^2.2	0.1864	0.4318	42	no study/comparison

Test for Residual Heterogeneity:

QE(df = 53) = 14676.6805, p-val < .0001

Test of Moderators (coefficients 2:8):

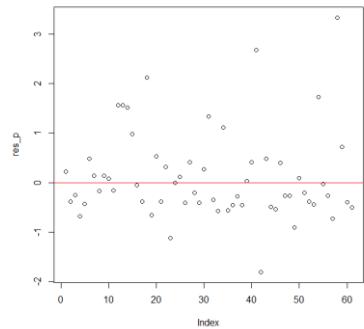
QM(df = 7) = 238.2578, p-val < .0001

Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub	
intrcpt	0.6517	0.2784	2.3410	0.0192	0.1061	1.1974	*
ss_ratelow	-0.3280	0.0243	-13.4936	<.0001	-0.3757	-0.2804	***
ss_ratemedium	-0.0312	0.0230	-1.3561	0.1751	-0.0763	0.0139	
regionamerica	0.7528	0.4139	1.8186	0.0690	-0.0585	1.5641	.
regionasia	-0.2651	0.5951	-0.4454	0.6561	-1.4315	0.9014	
regioneurope	0.7667	0.5864	1.3074	0.1911	-0.3827	1.9161	
regionoceania	-0.1773	0.5715	-0.3102	0.7564	-1.2974	0.9428	
locallab	0.7349	0.4974	1.4776	0.1395	-0.2399	1.7098	

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residuals:



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Publication Bias

Multivariate Meta-Analysis Model (k = 61; method: REML)

logLik	Deviance	AIC	BIC	AICc
-707.0406	1414.0811	1422.0811	1430.3913	1422.8219

Variance Components:

estim	sqrt	nlvls	fixed	factor
sigma^2.1	0.7691	0.8770	25	no
study_n				
sigma^2.2	0.1851	0.4302	39	no
study_n/comparison				

Test for Residual Heterogeneity:

QE(df = 59) = 24333.3336, p-val < .0001

Test of Moderators (coefficient 2):

QM(df = 1) = 10.9103, p-val = 0.0010

Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub					
intrcpt	1.1305	0.2032	5.5627	<.0001	0.7322	1.5289	***				
sqrt(vi)	-2.8240	0.8550	-3.3031	0.0010	-4.4997	-1.1483	***				
Signif. codes:	0	***	0.001	**	0.01	*	0.05	.	0.1	'	1

--

Global effect of the treatments

1 response SE df asymp.LCL asymp.UCL

overall **2.38** 0.404 Inf 1.71 3.32

Results are averaged over the levels of: ss_rate, region, local

Confidence level used: 0.95

Intervals are back-transformed from the log scale

--

Predict values (effect sizes, already transformed from log by the function *exp*):

mod	level	pred	ci.lb	ci.ub
rate	High	1.9188	1.111933	3.311496
rate	Low	1.382216	0.800662	2.386176
rate	Medium	1.859873	1.077876	3.209205
continent	Africa	1.9188	1.111933	3.311496
continent	America	4.07355	2.14824	7.724374
continent	Asia	1.472079	0.507846	4.26708
continent	Europe	4.130747	1.500586	11.37094
continent	Oceania	1.607097	0.603623	4.278768
condition	Field	1.9188	1.111933	3.311496
condition	Greenhouse	4.001525	1.439705	11.12186

ZINC

Multivariate Meta-Analysis Model ($k = 88$; method: REML)

logLik	Deviance	AIC	BIC	AICc
-1516.3230	3032.6460	3052.6460	3076.4663	3055.8344

Variance Components:

estim	sqrt	nlvls	fixed	factor
sigma^2.1	0.3637	0.6031	24	no study
sigma^2.2	0.0544	0.2332	45	no study/comparison

Test for Residual Heterogeneity:

QE(df = 80) = 7287.3183, p-val < .0001

Test of Moderators (coefficients 2:8):

QM(df = 7) = 3791.4413, p-val < .0001

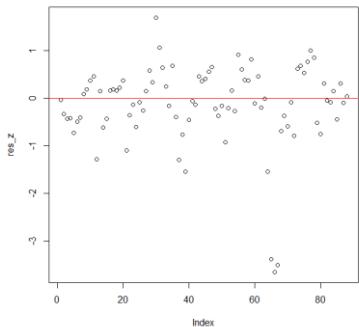
Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub	
intrcpt	0.7971	0.2709	2.9423	0.0033	0.2661	1.3281	**
ss_ratelow	-0.6882	0.0113	-60.8052	<.0001	-0.7104	-0.6660	***
ss_ratemedium	-0.3767	0.0104	-36.2256	<.0001	-0.3970	-0.3563	***
regionamerica	1.3879	0.3392	4.0919	<.0001	0.7231	2.0528	***
regionasia	-0.2484	0.4701	-0.5285	0.5972	-1.1699	0.6730	
regioneurope	-0.6060	0.4617	-1.3126	0.1893	-1.5109	0.2989	
regionoceania	0.2816	0.5314	0.5298	0.5963	-0.7601	1.3232	
locallab	0.7068	0.4045	1.7474	0.0806	-0.0860	1.4996	.

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

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Residuals



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Publication Bias

Multivariate Meta-Analysis Model (k = 88; method: REML)

logLik	Deviance	AIC	BIC	AICc
-3298.8203	6597.6407	6605.6407	6615.4581	6606.1345

Variance Components:

estim	sqrt	nlvls	fixed	factor
sigma^2.1	0.8778	0.9369	24	no
study_n				
sigma^2.2	0.1552	0.3939	45	no
study_n/comparison				

Test for Residual Heterogeneity:

QE(df = 86) = 32212.7322, p-val < .0001

Test of Moderators (coefficient 2):

QM(df = 1) = 222.9690, p-val < .0001

Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub					
intrcpt	1.5869	0.2084	7.6148	<.0001	1.1785	1.9954	***				
sqrt(vi)	-6.2739	0.4202	-14.9321	<.0001	-7.0974	-5.4504	***				
Signif. codes:	0	***	0.001	**	0.01	*	0.05	.	0.1	'	1

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Global effect of the treatment, proportional to moderators:

1 response SE df asympt.LCL asympt.UCL

overall **2.43** 0.38 Inf 1.79 3.3

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Predict values (already transformed from log scale):

mod	level	pred	ci.lb	ci.ub
rate	High	2.219	1.3048	3.7738
rate	Low	1.115031	0.655657	1.896259
rate	Medium	1.522614	0.895343	2.589346
continent	Africa	2.219	1.3048	3.7738
continent	America	8.891029	5.788255	13.65704
continent	Asia	1.730921	0.735502	4.073532
continent	Europe	1.210556	0.581694	2.519271
continent	Oceania	2.940703	1.199948	7.206755
condition	Field	2.219	1.3048	3.7738
condition	Greenhouse	4.499249	1.959086	10.333

COPPER

Multivariate Meta-Analysis Model ($k = 98$; method: REML)

logLik	Deviance	AIC	BIC	AICc
-1600.1672	3200.3343	3220.3343	3245.3324	3223.1191

Variance Components:

estim	sqrt	nlvls	fixed	factor
sigma^2.1	0.0967	0.3110	28	no study
sigma^2.2	0.3442	0.5867	52	no study/comparison

Test for Residual Heterogeneity:

QE(df = 90) = 9102.8543, p-val < .0001

Test of Moderators (coefficients 2:8):

QM(df = 7) = 14121.1364, p-val < .0001

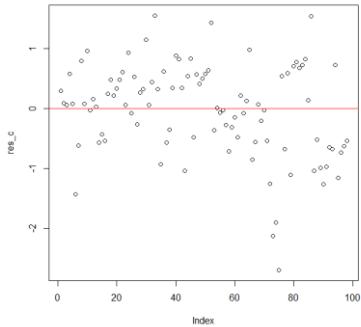
Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub	
intrcpt	1.0797	0.2513	4.2973	<.0001	0.5873	1.5722	***
ss_ratelow	-1.1101	0.0097	-114.9971	<.0001	-1.1290	-1.0911	***
ss_ratemedium	-0.7129	0.0082	-87.0519	<.0001	-0.7290	-0.6969	***
regionamerica	0.8036	0.3069	2.6186	0.0088	0.2021	1.4050	**
regionasia	0.0178	0.3304	0.0539	0.9571	-0.6297	0.6653	
regioneurope	-0.3536	0.4168	-0.8484	0.3962	-1.1706	0.4633	
regionoceania	0.1165	0.4583	0.2541	0.7994	-0.7818	1.0147	
locallab	0.3766	0.2780	1.3548	0.1755	-0.1682	0.9214	

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

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Residuals:



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Publication Bias (copper)

Multivariate Meta-Analysis Model (k = 98; method: REML)

logLik	Deviance	AIC	BIC	AICc
-8606.1927	17212.3854	17220.3854	17230.6428	17220.8250

Variance Components:

estim	sqrt	nlvls	fixed	factor
sigma^2.1	0.5400	0.7348	28	no
study_n				
sigma^2.2	0.3678	0.6065	52	no
study_n/comparison				

Test for Residual Heterogeneity:

QE(df = 96) = 38199.9142, p-val < .0001

Test of Moderators (coefficient 2):

QM(df = 1) = 110.7854, p-val < .0001

Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub
intrcpt	0.1113	0.1777	0.6260	0.5313	-0.2371	0.4596
sqrt(vi)	5.3374	0.5071	10.5255	<.0001	4.3435	6.3313
Signif. codes:	0	***	0.001	**	0.01	*
					0.05	.
					0.1	'
					1	

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Global effect of the treatment (back transformed from log scale)

1 response SE df asym.LCL asym.UCL

overall **2.06** 0.238 Inf 1.64 2.58

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Predict values:

mod	level	pred	ci.lb	ci.ub
rate	High	2.943796	1.799124	4.817234
rate	Low	0.970116	0.592799	1.587596
rate	Medium	1.443119	0.881907	2.361464
continent	Africa	2.943796	1.799124	4.817234
continent	America	6.5751	4.52518	9.55364
continent	Asia	2.9967	1.805944	4.972585
continent	Europe	2.066997	1.076928	3.96728
continent	Oceania	3.307475	1.560227	7.011409
condition	Field	2.943796	1.799124	4.817234
condition	Greenhouse	4.290105	2.263335	8.131805