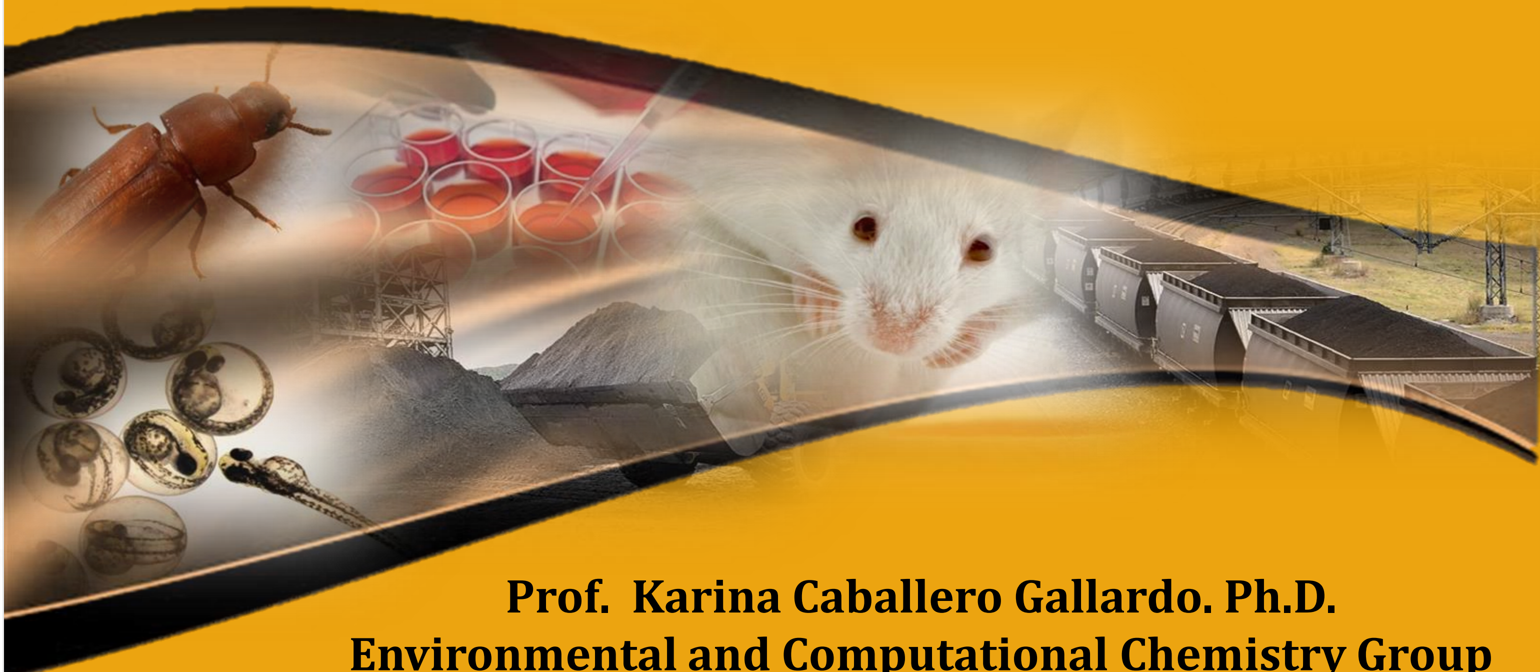
Environmental health perspectives on coal pollution: a view from different toxicological models



Prof. Karina Caballero Gallardo. Ph.D.
Environmental and Computational Chemistry Group
University of Cartagena, Cartagena, Colombia

We are Concerned about Environmental Health

Chemosphere. 2015. 138: 837-846.

Chemical and toxicological characterization of sediments along a Colombian shoreline impacted by coal export terminals.

Caballero-Gallardo K[1], Guerrero-Castilla A[1], Johnson-Restrepo B[2], de la Rosa J[3], Olivero-Verbel J[4].

Toxicol Appl Pharmacol. 2016. 294: 11-20.

Mice housed on coal dust-contaminated sand: A model to evaluate the impacts of coal mining on health.

Caballero-Gallardo K[1], Olivero-Verbel J[2].

Metallomics. 2018. 10(3): 463-473.

Embryonic exposure to an aqueous coal dust extract results in gene expression alterations associated with the development and function of connective tissue and the hematological system, immunological and inflammatory disease, and cancer in zebrafish.

Caballero-Gallardo K[1], Wirbisky-Hershberger SE[2], Olivero-Verbel J[1], de la Rosa J[3], Freeman JL[2].

Submitted to Environmental Research

Transgenerational effects of coal dust on *Tribolium castaneum*, Herbst

Alcala-Orozco, M., Caballero-Gallardo K., Olivero-Verbel J

COAL MINING IN COLOMBIA

Oil Refinery... Santander

Oil Refinery... Bolivar

Coal mining... Guajira

Coal mining... Cesar

Gold mining... Amazon

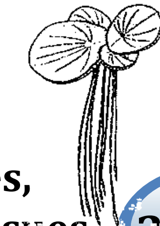
Gold mining... Choco

Gold mining... Bolivar

Our Researches

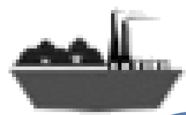
Damage to DNA
Cabarcas-Montalvo et al.

2012



Chlorosis, reduction in the size of leaves, abscission of leaves and roots, necrotic tissues
Coronado-Posada et al.

2013



Presence of PAH and heavy metals in marine Sediments of Coal export terminals
Caballero-Gallardo et al.

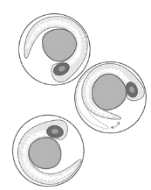
2015

Timeline – 10 Years

Environmental and Computational Chemistry Group

Coal dust induces molecular, cellular and histopathological changes in mice
Caballero-Gallardo et al.

2016



2018

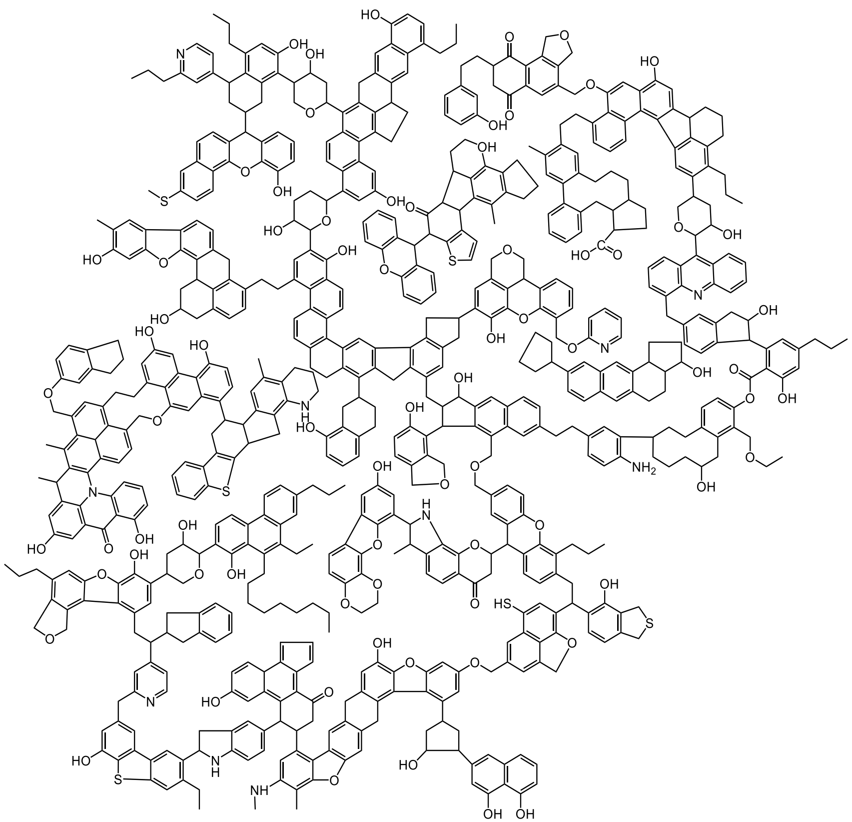
Transcriptome results signifying functions and systems to target in future studies
Caballero-Gallardo et al.

Today

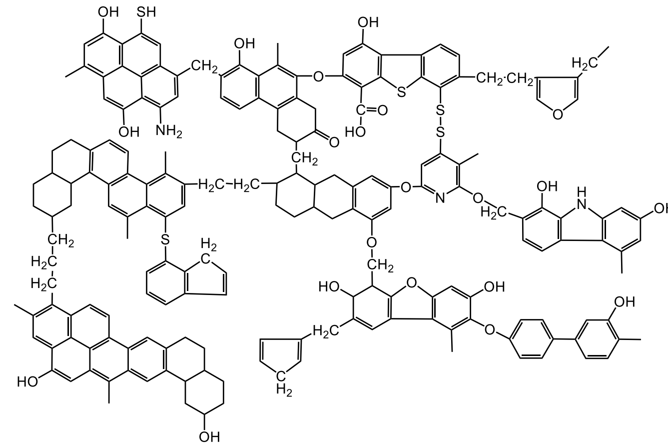
Coal

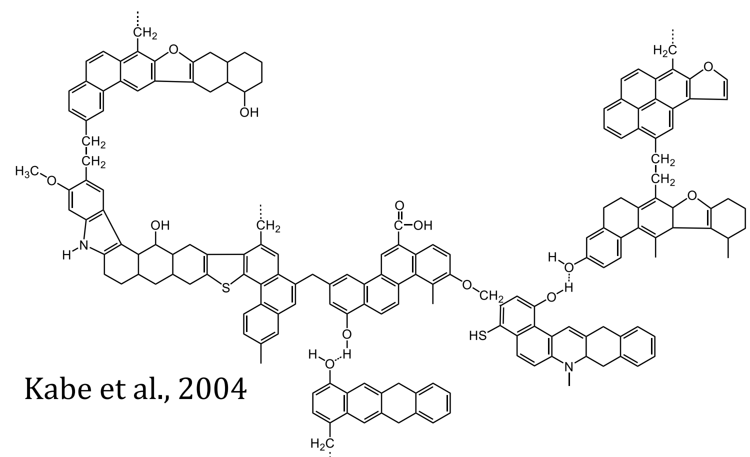
Coal molecular structure model

Shinn



Wiser





Kabe et al., 2004

Solomon

The phases of coal mining

Exploration

Exploitation

Transport

Coal dust

Thomas (2013).

Coal dust particles are formed when two pieces of mineral crush or get in close contact

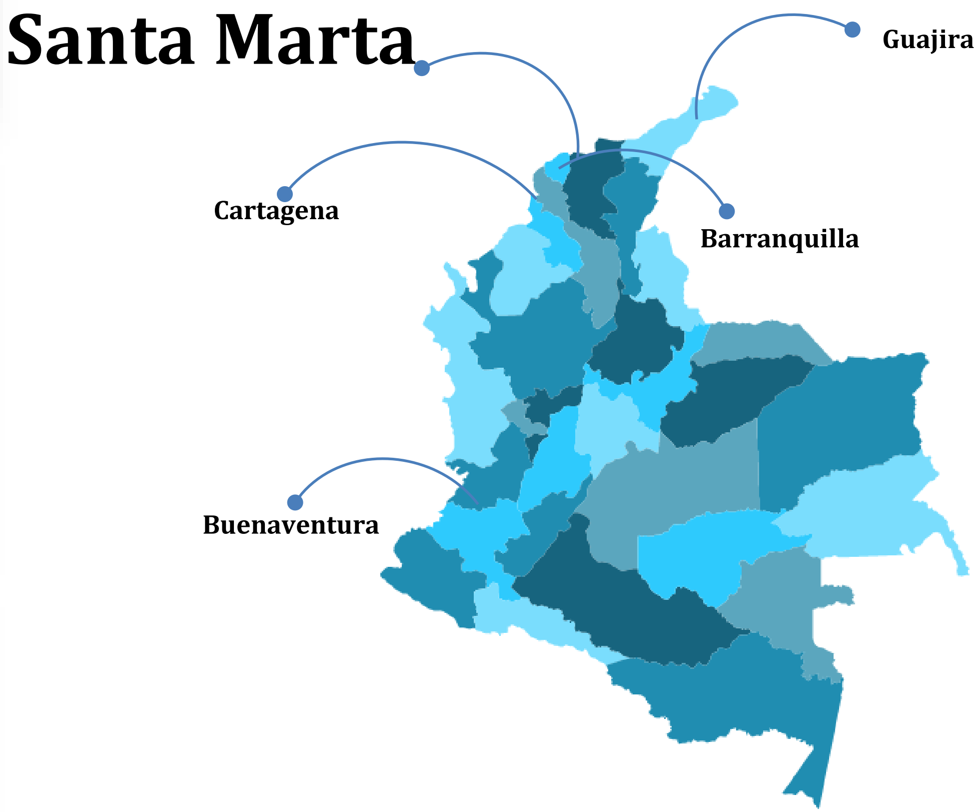
1 PAHs

2 Heavy metals

3 Dust particles

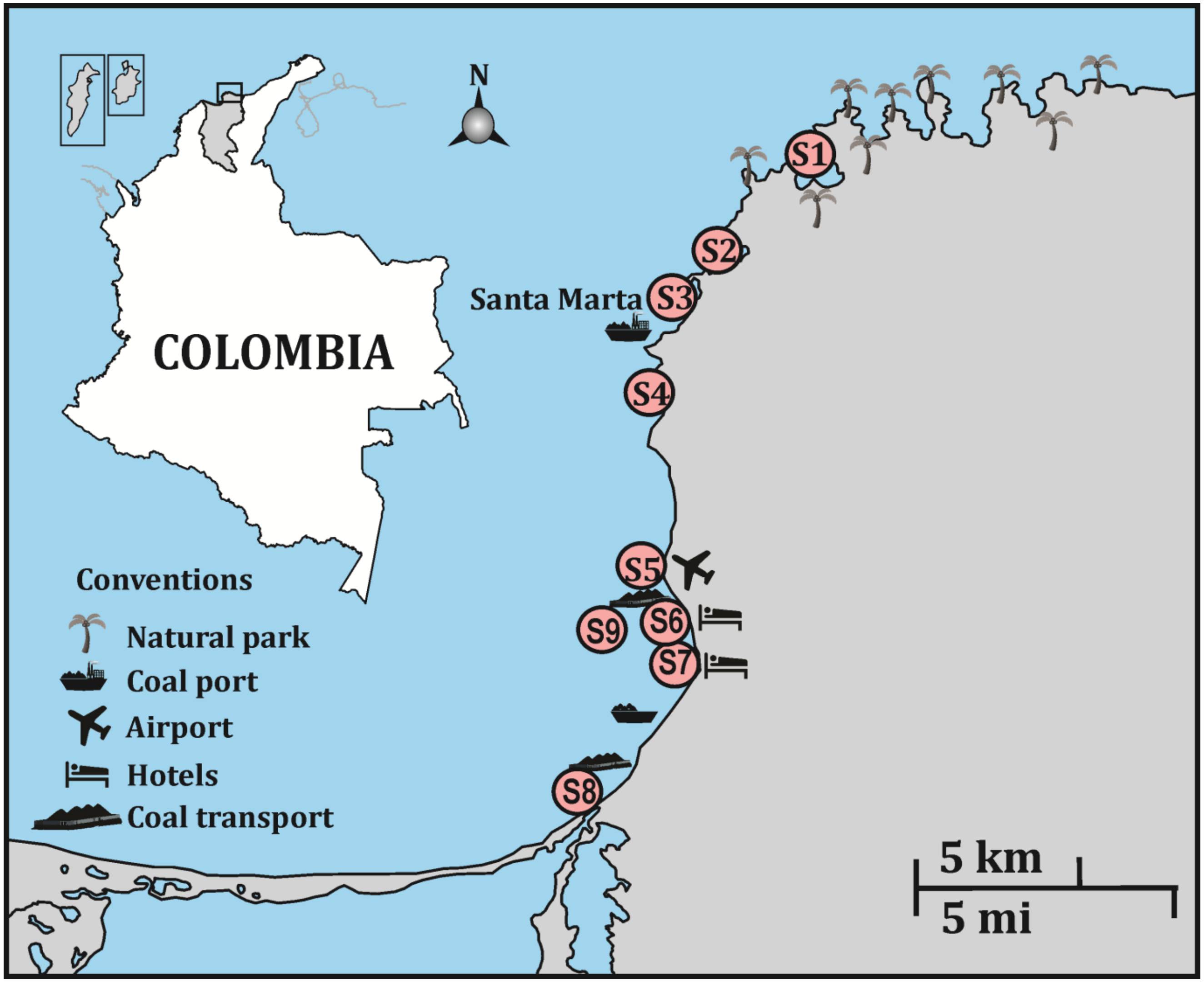
Coal Port in Santa Marta, Colombia

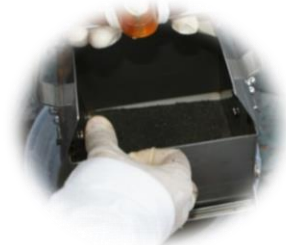
Colombian Ports for Coal



Caballero-Gallardo et al., 2015

Study area



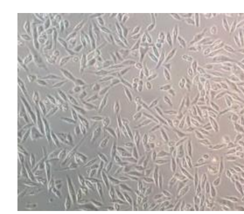


Marine sediments

ICP-MS
Trace elements 1

GC-MS
PAHs 2

Cell cultures (HepG2)



3

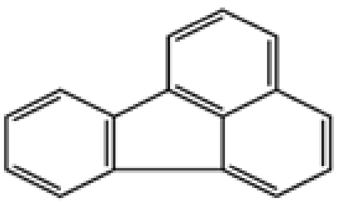
Caballero-Gallardo et al., 2015

Trace element concentrations (µg/g, dry weight) in sediments from Santa Marta shoreline, Colombia, compared to marine Sediment Quality Standards

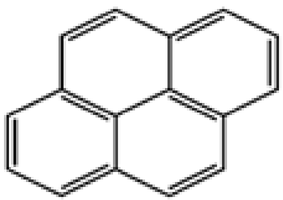
Station/index name		Cd	Cr	Cu	Pb	Zn	As
S1		0.1	30.5	**13.3**	6.3	46.8	2.7
S2		0.1	**141.2**	**22.0**	6.6	**59.2**	3.1
S3		0.2	48.8	9.77	**11.0**	**69.7**	3.2
S4		0.1	44.9	4.16	6.6	54.9	3.0
S5		0.1	39.7	**29.9**	7.1	53.7	2.5
S6		0.1	38.8	3.5	6.5	48.9	2.8
S7		0.1	33.7	4.0	5.7	58.3	2.4
S8		0.2	**64.9**	**21.4**	**10.0**	**89.3**	7.4
S9		0.1	**67.1**	**22.1**	**12.2**	**106.0**	**11.2**
Marine sediment pollution index (MSPI) Shin y Lam (2001)	Percentile 0–20	0.6	2.0	3.0	3.3	15.4	7.0
	Percentile 21–40	1.0	5.0	6.0	5.0	34.0	8.0
	Percentile 41–60	1.5	9.2	12.0	8.0	57.0	10.2
	Percentile 61–80	2.9	19.6	30.6	18.2	101.6	21.0
	Percentile 81–100	8.0	63.0	191.0	69.0	507.0	58.0

The sediment quality based on MSPI, as follows: MSPI 0-20: sediment in excellent condition; MSPI 21-40: sediment in good condition; MSPI 41-60: sediment in average condition; MSPI 61-80: sediment in poor condition; MSPI 81-100: sediment in bad condition.

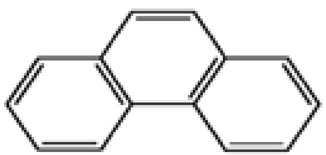
PAHs in marine sediments



Fluoranthene
FL

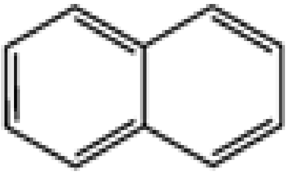


Pyrene
Pyr



Phenanthrene
Phe

Coal waste materials from coal areas
Ribeiro et al., 2012



Naphtalene
Nap

Coal cargo transport

Various types of coal
(Achten y Hofmann, 2009;
Van Kooten et al., 2002;
Tripp et al., 1981).
S9, S8 y S7

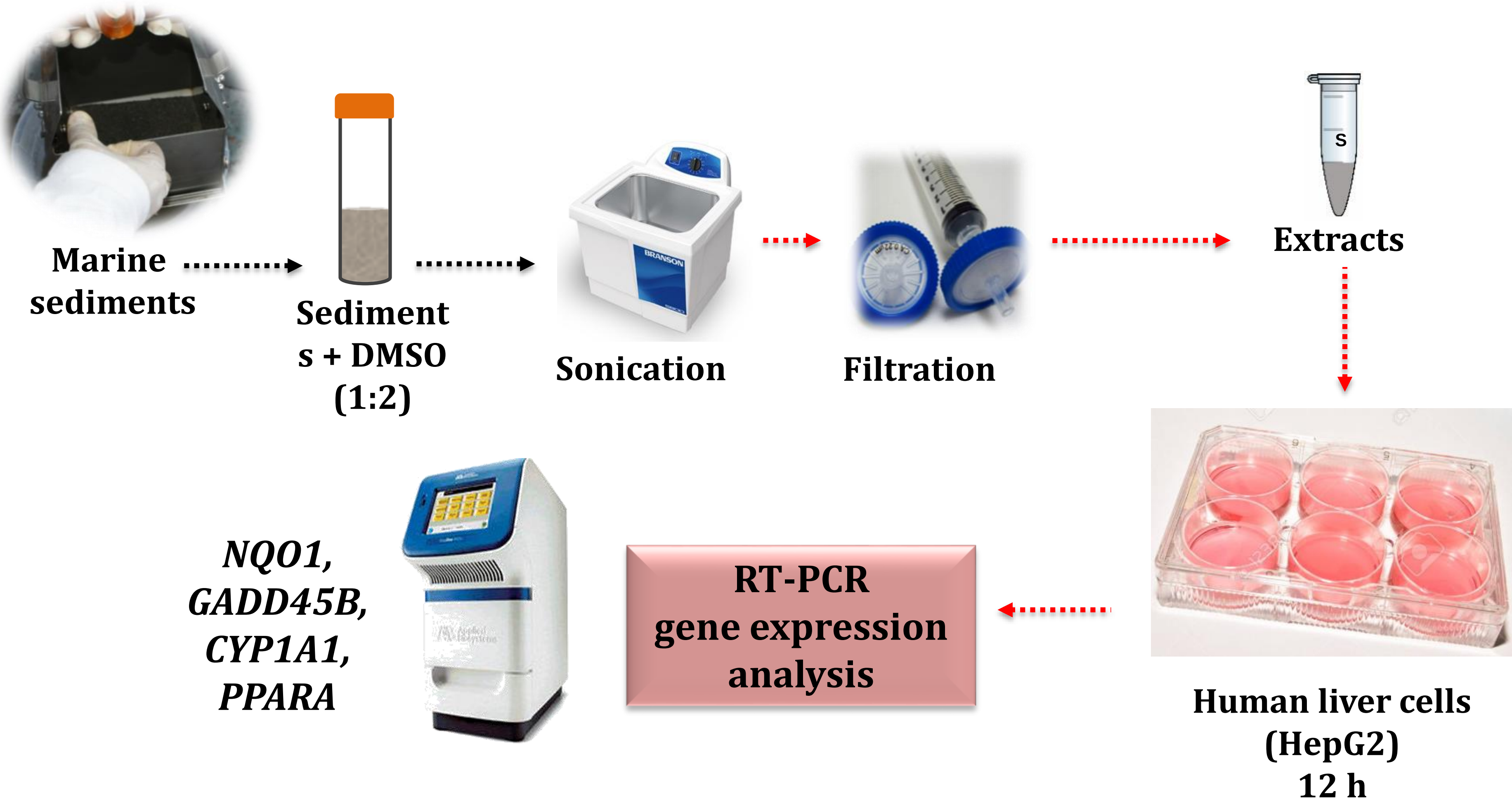
Coal pollution in beaches

Metals
PAHs

Are those chemicals biologically active?

Do they represent a problem for the biota or maybe the humans?

Biological experiments with sediment extracts



Relative quantification of mRNA of *CYP1A1* (Cytochrome P450, family 1, subfamily A, polypeptide 1), *NQO1* (NAD(P)H dehydrogenase quinone 1), *GADD45B* (DNA damageinduciblegene 45 b) and *PPARA* (Peroxisome proliferator-activated receptor alpha) in HepG2 Cells treated with 1% marine sediment extracts

	RELATIVE QUANTIFICATION OF mRNA							
	CYP1A1		NQO1		GADD45B		PPARA	
SAMPLE	HMBS	B2M	HMBS	B2M	HMBS	B2M	HMBS	B2M
C-	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ±0.1	1.0 ± 0.2	1.0 ± 0.2
CE (500 ppm)	176.4 ± 23.0*	189.9 ± 31.8*	1.9 ± 0.0	2.6 ± 0.6	6.8 ± 1.7*	7.3 ± 1.8*	1.1 ± 0.3	1.2 ± 0.4
CE (1000 ppm)	307.2 ± 40.5*	231.9 ± 73.0*	3.9 ± 0.4*	4.0 ± 0.1*	30.2 ± 9.5*	19.0 ± 4.0*	1.6 ± 0.9	0.8 ± 0.0
S1	3.6 ± 0.4	2.7 ± 0.1	1.8 ± 0.4	1.3 ± 0.1	1.4 ± 0.3	1.0 ± 0.0	0.6 ± 0.1	0.5 ± 0.0
S2	2.6 ± 0.9	1.6 ± 0.2	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.2	0.9 ± 0.2	0.7 ± 0.2	0.4 ± 0.1
S3	**61.5 ± 13.1***	**36.6 ± 2.4***	2.1 ± 0.2	1.7 ± 0.0	1.4 ± 0.2	0.8 ± 0.1	0.6 ± 0.1	0.4 ± 0.1
S4	1.2 ± 0.2	1.7 ± 0.3	1.0 ± 0.3	1.2 ± 0.1	0.7 ± 0.3	0.8 ± 0.3	0.5 ± 0.1	0.8 ± 0.2
S5	0.5 ± 0.1	1.0 ± 0.1	1.4 ± 0.1	2.7 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.8 ± 0.1	1.6 ± 0.1
S6	1.1 ± 0.4	1.5 ± 0.5	3.3 ± 0.9*	3.4 ± 0.4*	0.1 ± 0.0	0.1 ± 0.0	1.4 ± 0.2	1.8 ± 0.9
S7	1.1 ± 0.3	1.3 ± 0.4	4.3 ± 1.3*	3.0 ± 0.4*	0.1 ± 0.0	0.2 ± 0.0	0.9 ± 0.1	1.6 ± 0.2
S8	2.2 ±0.2	3.3 ± 0.7	2.4 ±0.1*	3.5 ± 0.5*	0.1 ± 0.0	0.2 ± 0.0	0.8 ± 0.1	1.2 ± 0.3
S9	**11.9 ± 4.5***	**11.4 ± 3.2***	3.7 ±0.9*	3.6 ±0.1*	0.2 ± 0.0	0.2 ± 0.1	1.0 ± 0.1	1.1 ± 0.2

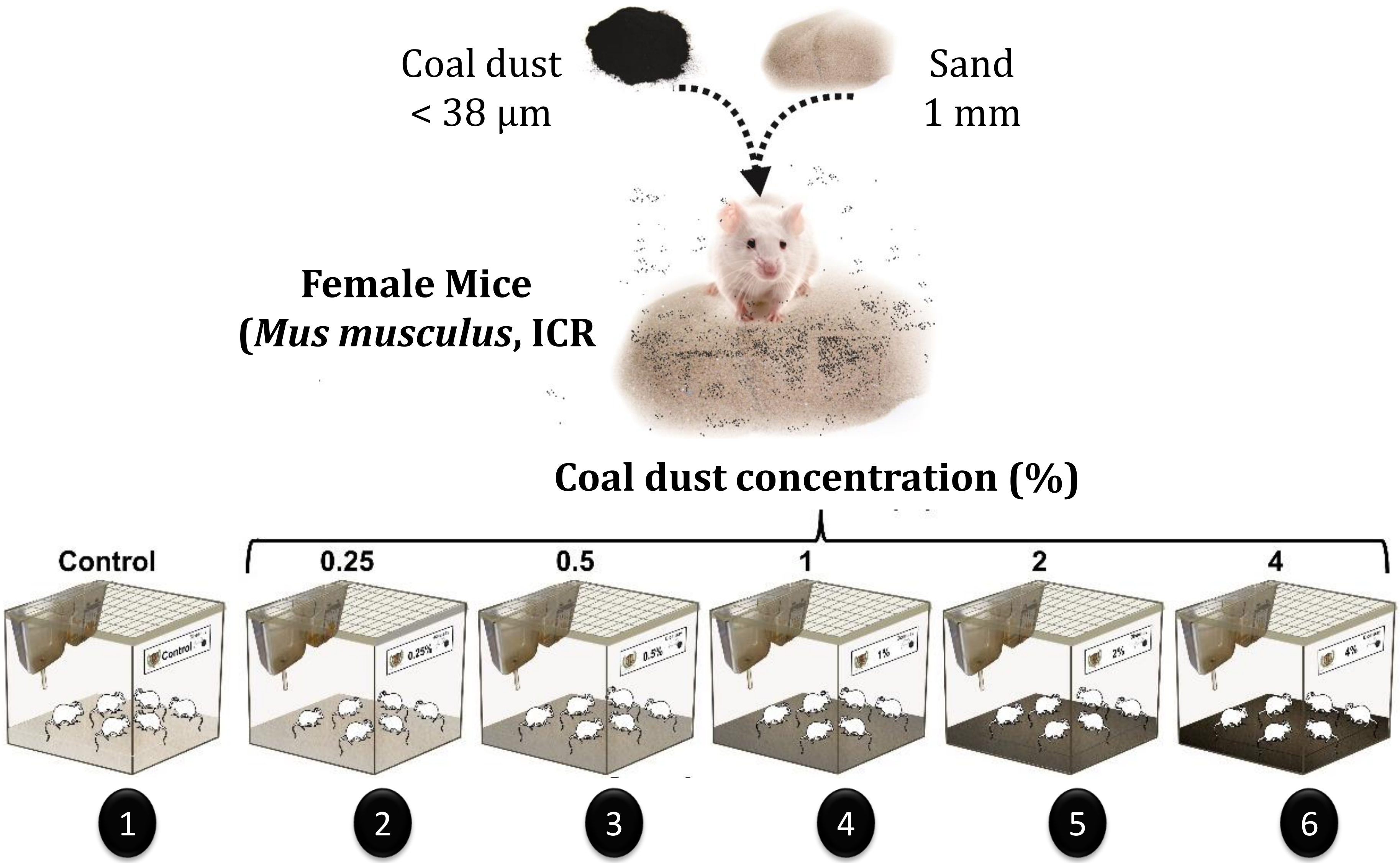
Gene expression was normalized with HMBS and B2M. C-, Negative control; CE, Coal Extract (Positive control); S1-S9, marine sediment extracts.* Significant difference ($P < 0.05$) compared to the Negative control.

Sediments from coal ports

Pollutans → Biologically Active → Biota → Humans


Is it possible that these effects also occur in the soil, when biota organisms have direct contact with coal dust particles?

Model of coal dust exposure in sand



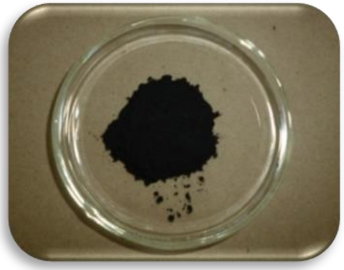
For eight weeks exposure

Coal dust <38 µm

Coal sample (Cesar, Colombia)

Crushed with a pestle and mortar

Sieved (20/40/60/100/400)



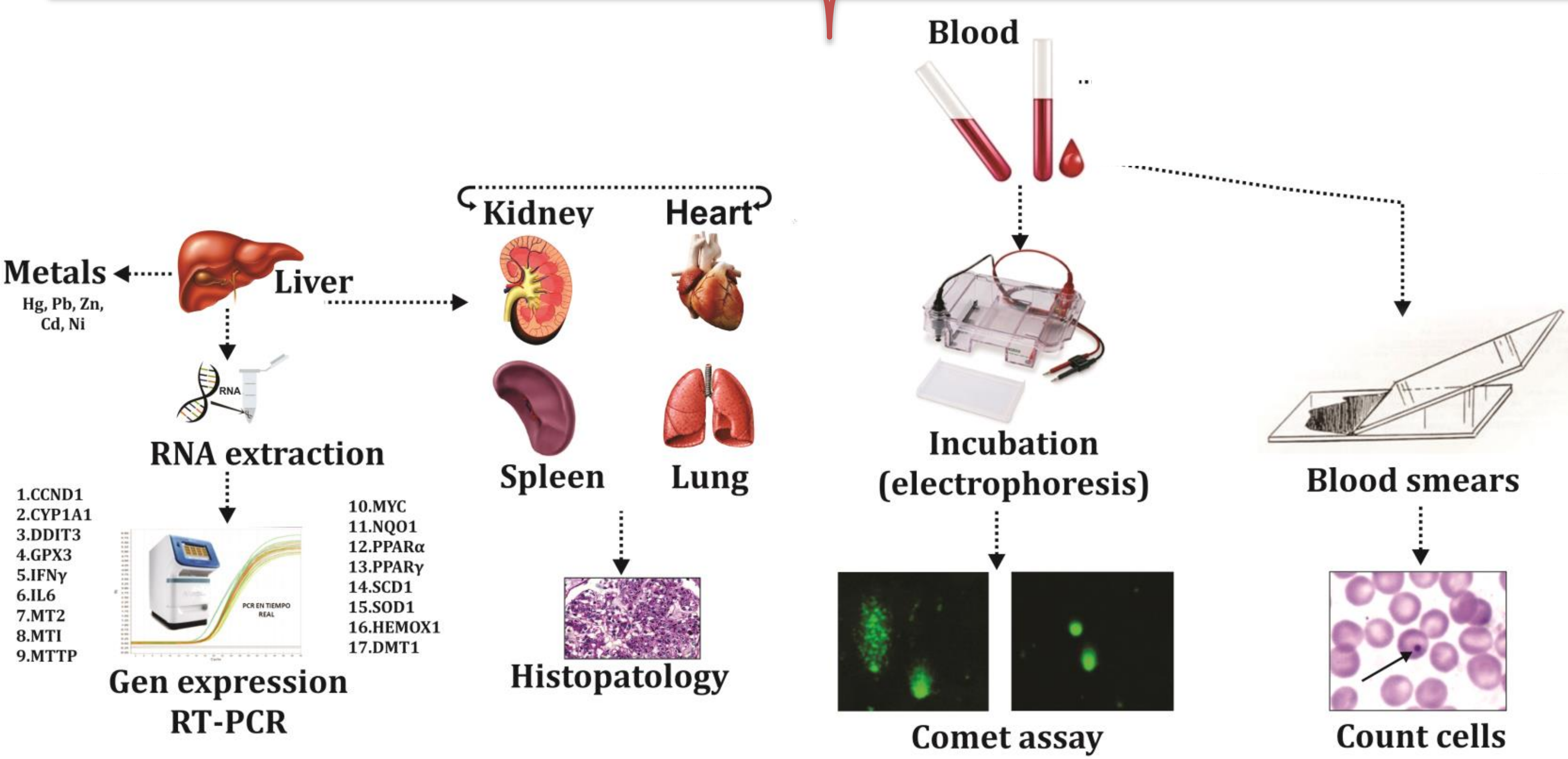
Coal dust <38 µm

The routes of exposure

Coal dust ingestion **Dermal contact** **Coal dust inhalation**

Caballero-Gallardo et al., 2016

Blood and tissue collection



Caballero-Gallardo et al., 2016

Metals concentrations (µg/g) in hepatic tissue

Metal	n	Control	2 %	4 %
Hg	6	<DL	<0.0004	<0.0004
Pb	5	<DL	<0.038	<0.038
Ni	6	0.07±0.003	0.07±0.001	0.09±0.001*
Cd	6	0.07±0.001	0.06±0.001	0.07±0.002
Zn	6	78.92±0.10	80.22±0.20*	87.74±0.59*

*. Significant difference (*P*<0.05) when compared to non-exposed group (sand without coal dust).

Bats

79.67±11.8 µg/g
Zocche et al. (2010)
Brazil

Amphibians

87.4 ± 6.4 µg/g
Zocche et al. (2013)
Brazil

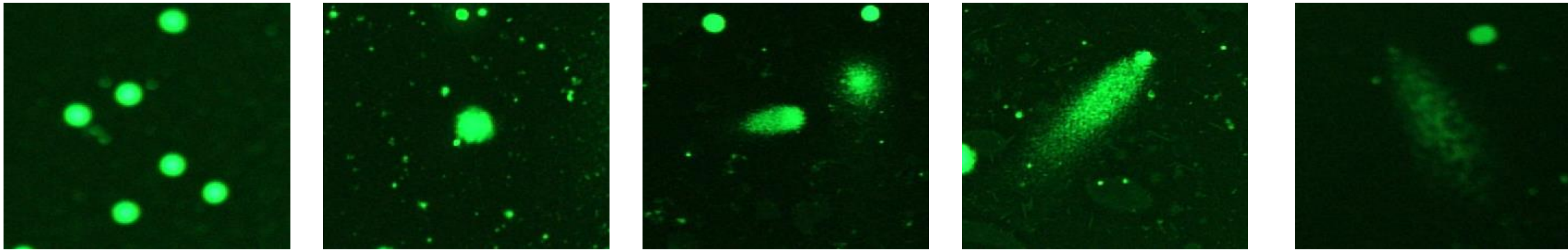
Mice

16.73 ± 4.73 µg/g
Guerero-Castilla et al. (2014)
Colombia

Caballero-Gallardo et al., 2016

Comet assay

Normal cell **Level I** **Level II** **Level III** **Level IV**



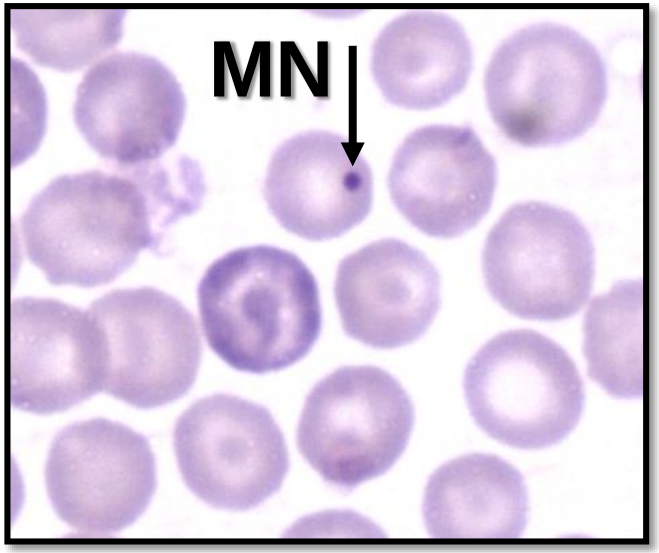
Group (Coal dust % in sand)	n	GDI± SD	F_{2+3+4}± SD
0	6	0.96±1.02	0.09±0.16
0.25	6	2.72±4.29	0.07±0.16
0.5	6	6.27±6.80	0.53±1.30
1	5	6.90±0.51	0.63±0.81
2	6	12.98±3.45*	2.50±0.11*
4	6	16.87±5.13*	5.38±1.00*

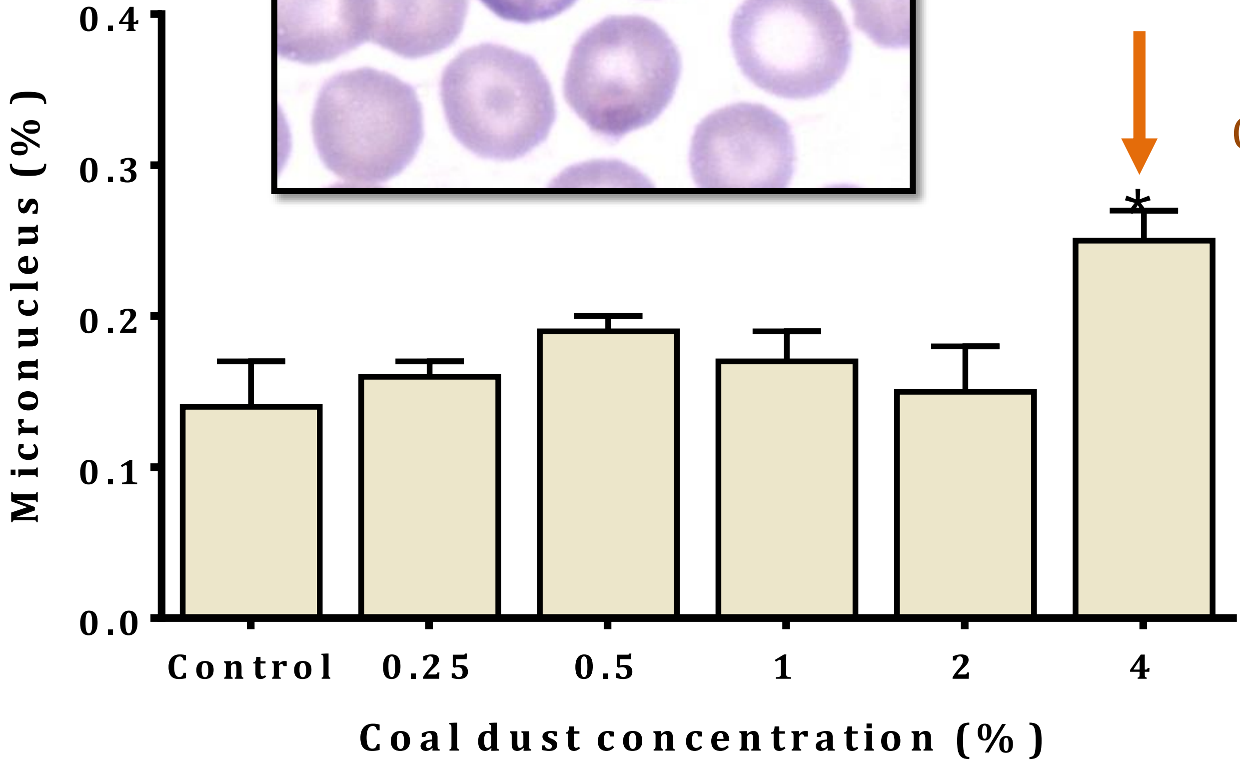
*. Significant differences (p<0.05) compared to control group (sand without coal dust)

Organisms monitoring can be used to predict possible genotoxic effects on humans

Caballero-Gallardo et al., 2016

Micronucleus in blood smears





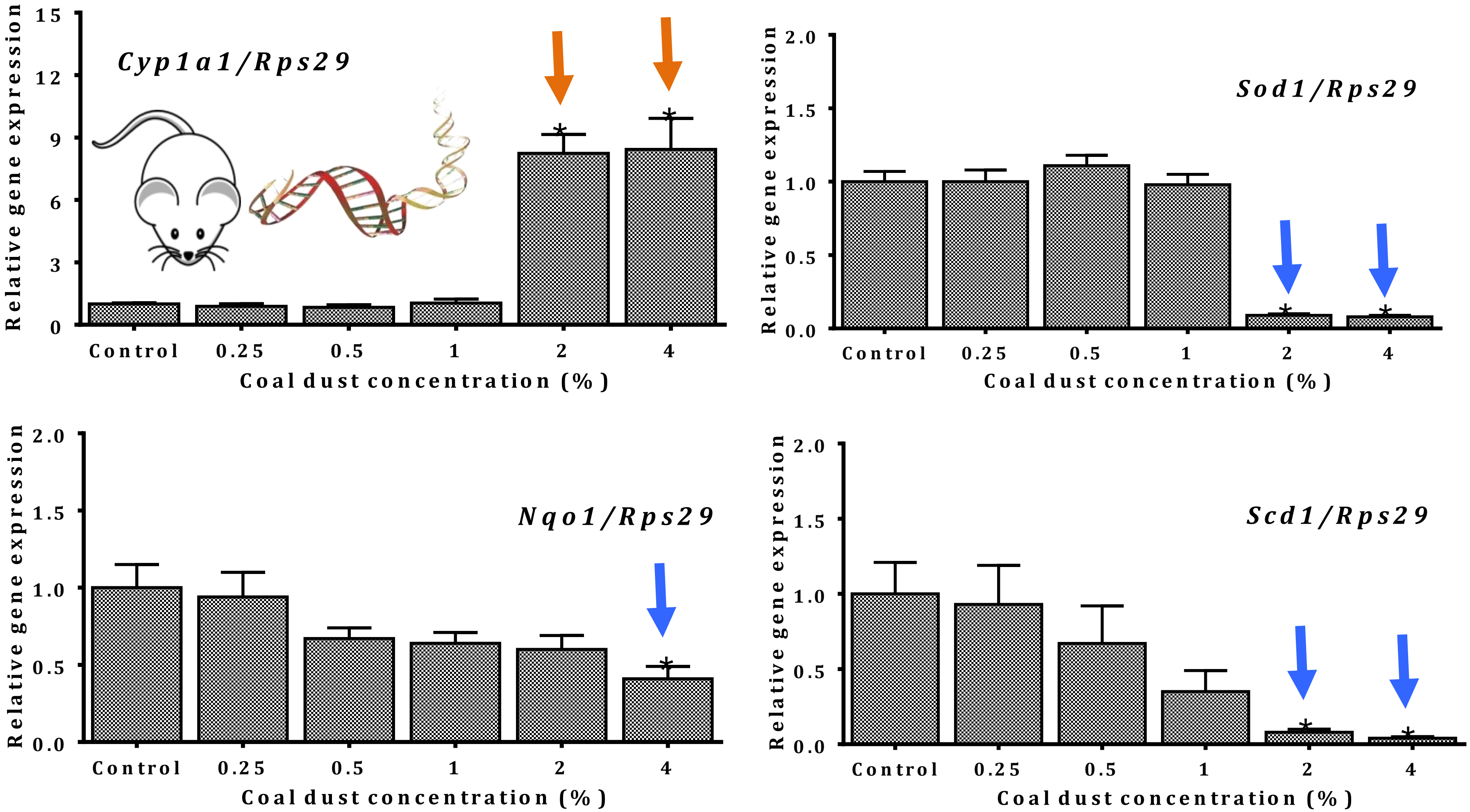
Rodents
Cabarcas-Montalvo et al. 2012

Mining workers
León-Mejía et al. 2011

*. Significant difference (P<0.05) when compared to control group.

Caballero-Gallardo et al., 2016

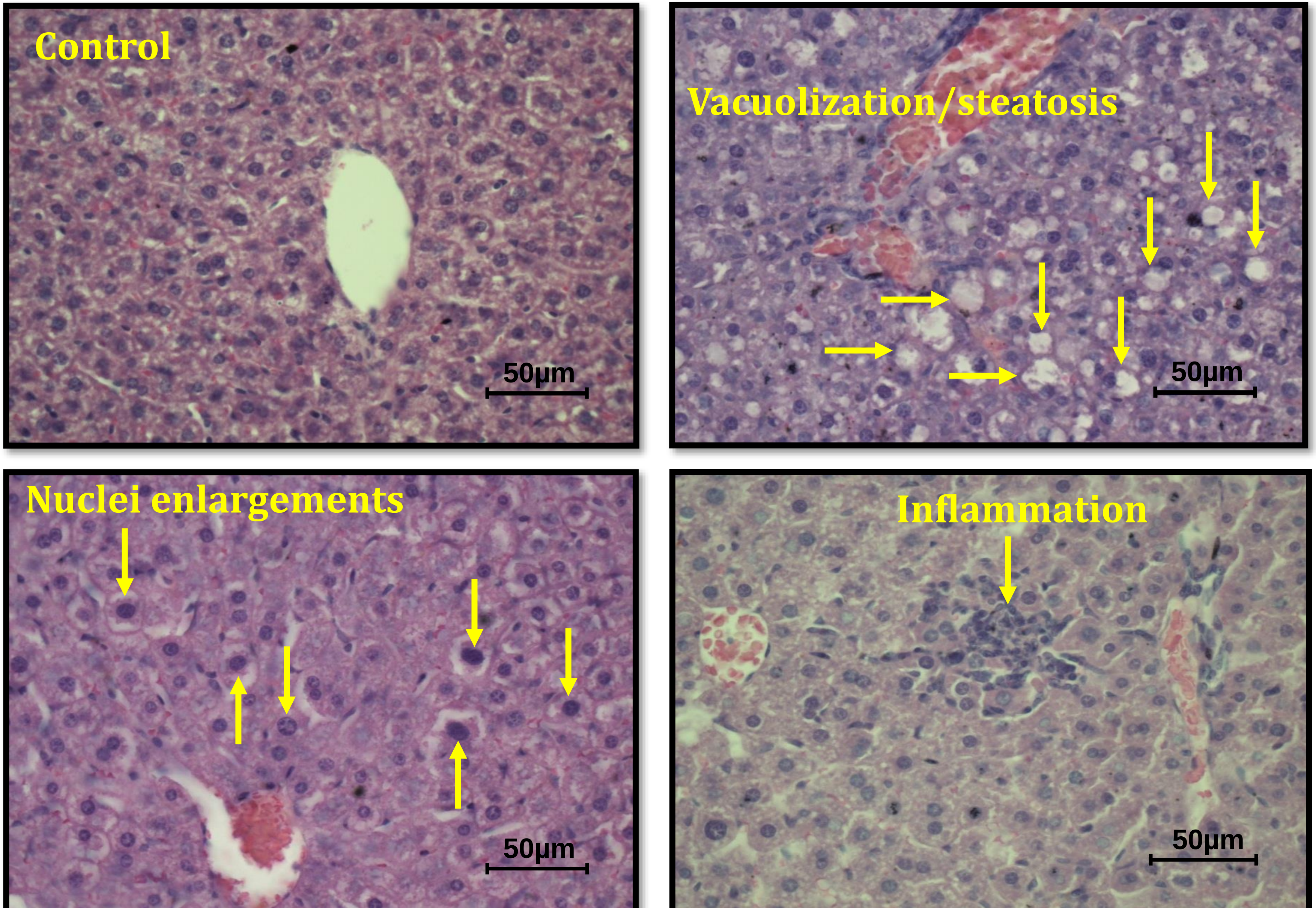
Hepatic gene expression profiles

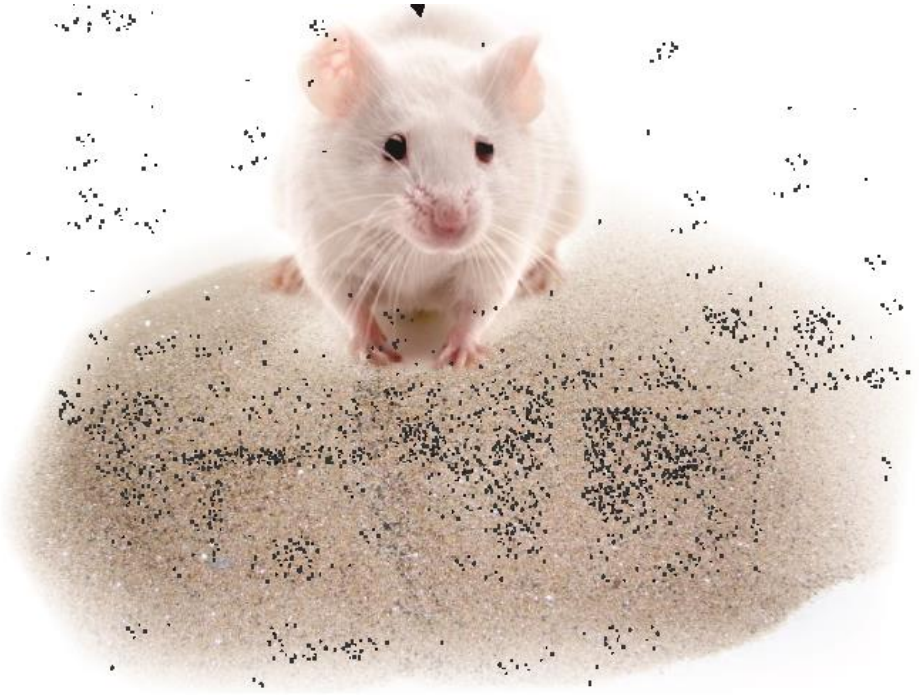


*Significant difference (p<0.05) when compared to control. ***Rps29***: ribosomal protein S29. ***Cyp1a1***: Cytochrome P450, family 1, subfamily A, polypeptide 1. ***Sod1***: Superoxide dismutase [Cu-Zn]. ***Nqo1***: NAD(P)H dehydrogenase quinone 1. ***Scd1***: Stearoyl-CoA desaturase.

Caballero-Gallardo et al., 2016

Morphological alterations in the liver tissue of mice after 8 weeks of exposure to sand contaminated with coal dust. Stain H&E (40×)





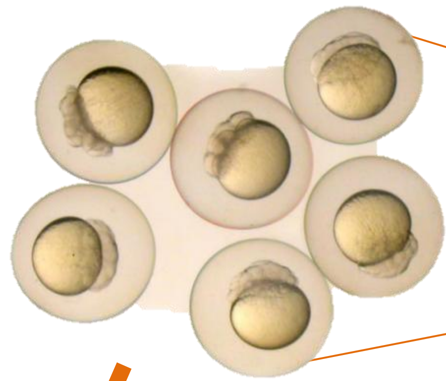
Mice exposed to coal dust under laboratory conditions, showed several toxicological effects at the molecular, cellular and tissue level, similar to those found in some organisms living near coal mining areas

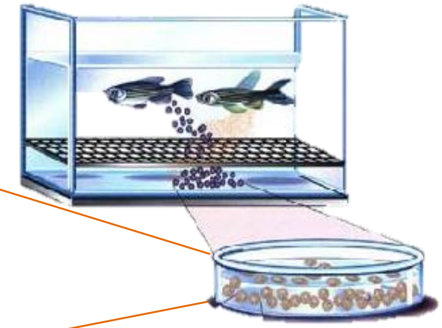
Caballero-Gallardo et al., 2016


Metals
PAHs

Toxic effects of an aqueous extract of coal dust in the embryonic development on Zebrafish (*Danio rerio*)

Aqueous extract of coal dust <38 µm





Control
1 ppm
0.1 ppm
1 ppm
10 ppm
100 ppm
1000 ppm

From 1 to 72 hpf

Mortality, alterations in hatching and morphological assessment

Transcriptomic profile

Microarrays and qPCR validation

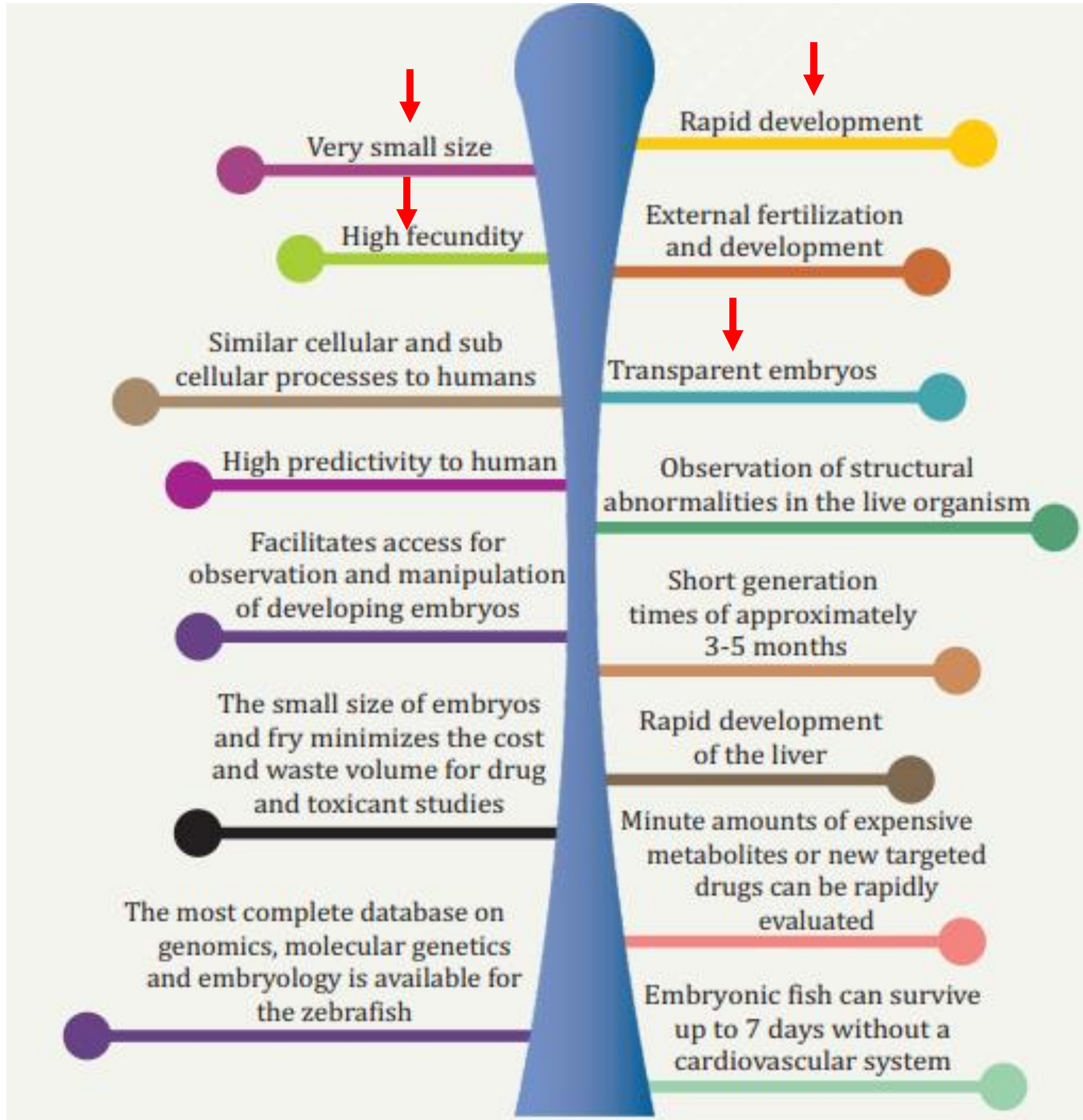
Caballero-Gallardo et al., 2018

Zebrafish: Advantages as a toxicological model

Order: Cypriniformes
Family: Cyprinidae

Zebrafish (*Danio rerio*)

Zebrafish are genetically similar to humans with approximately 70%



Howe et al., 2013; Yu et al., 2008

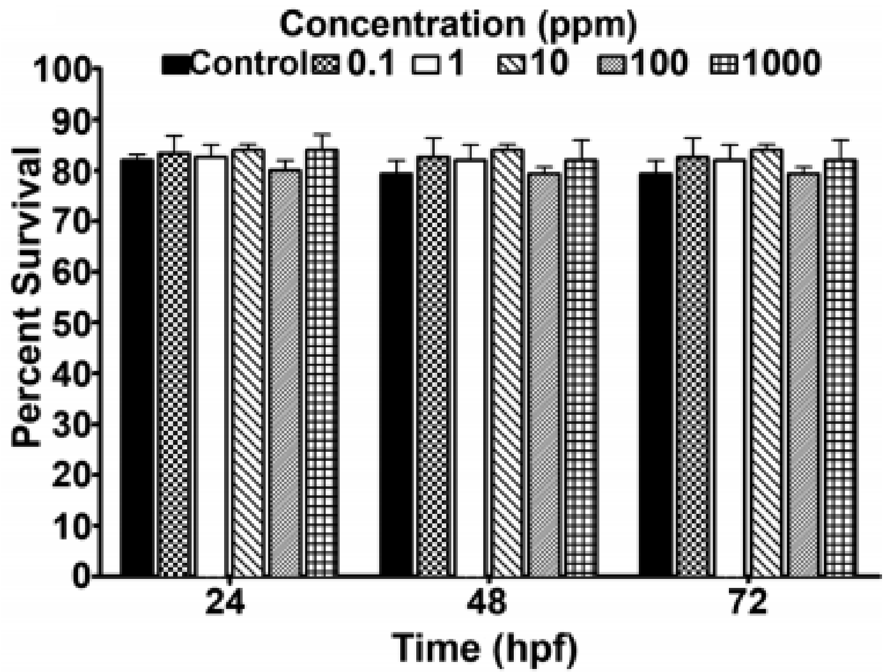
Trace element concentrations (ppb) in the aqueous coal dust extract, as well as Milli-Q water and coal dust (<38 μm) used for extraction

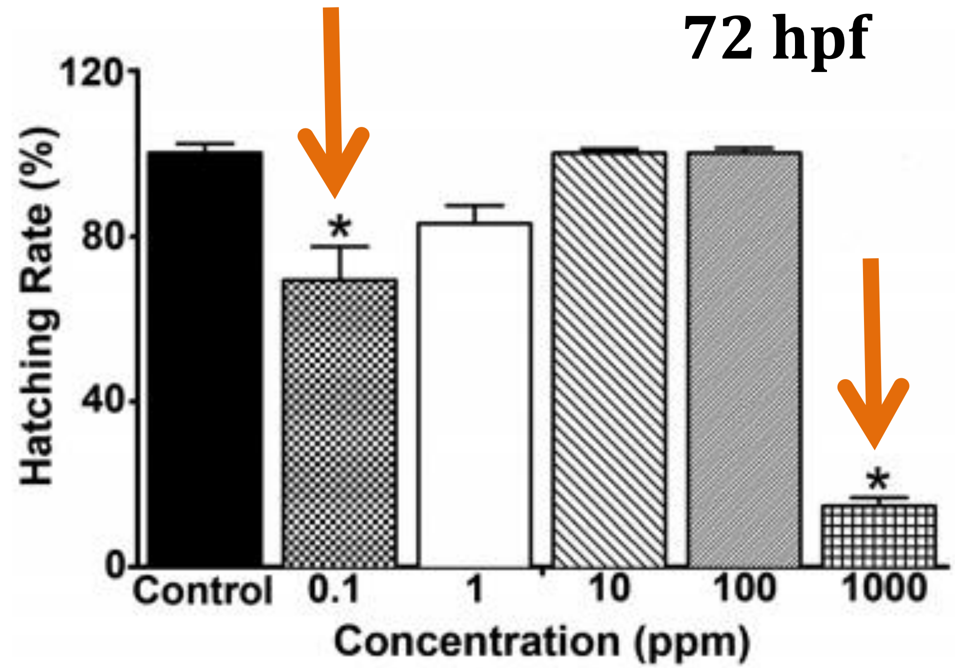
	Concentration (ppb; μg L^{-1})			
Elements	Aqueous coal dust extract	Milli-Q water	Δ concentration	Coal dust < 38 μm[27]
Sr[d]	600 ± 3[a]	0	600	34 910
Zn[c]	88 ± 0	3	85	8990
Ba[d]	67 ± 0	1	66	57 200
As[b]	20 ± 1	0	20	4380
Cu[c]	18 ± 2	1	17	4260
Se[b]	12 ± 0	0	12	1280
Li[d]	9.4 ± 0.0	0.0	9.4	750
Ni[c]	4.4 ± 0.1	0.0	4.4	870
Sb[d]	2.5 ± 1.6	0.0	2.5	90
Rb	2.4 ± 0.0	0.1	2.3	160
Co[d]	2.2 ± 0.0	0.0	2.2	550
Cr[c]	1.3 ± 0.0	0.8	0.5	1070
Mo[b]	0.9 ± 0.0	0.0	0.9	900
Tl[f]	0.7 ± 0.0	0.0	0.7	190
V[c]	0.5 ± 0.0	0.2	0.3	1170
Sc	0.5 ± 0.0	0.1	0.4	10
Pb[b]	0.4 ± 0.3	0.0	0.4	710
Bi	0.6 ± 0.2	0.0	0.6	20
Cs	0.2 ± 0.0	0.0	0.2	10
Be	0.2 ± 0.1	0.0	0.2	50
Zr	0.1 ± 0.0	0.0	0.1	770
Ge[d]	0.1 ± 0.0	0.0	0.1	80
U[e]	0.1 ± 0.0	0.0	0.1	40
Cd[b]	0.1 ± 0.0	0.0	0.1	40
Sn[f]	0.1 ± 0.0	0.0	0.1	110
Ga	0.1 ± 0.0	0.0	0.1	290

57% of the trace elements identified in the coal dust were found in the leachate.

Caballero-Gallardo et al., 2018

Percent survival and alterations in hatching of zebrafish exposed to aqueous coal extract from 1 to 72 hpf





*. Significant difference ($p<0.05$) when compared to control group.

Caballero-Gallardo et al., 2018

Transcriptomic results of zebrafish exposed to aqueous coal extract showed alterations in 77, 61, and 1376 genes differentially expressed in all groups evaluated (1, 10, and 100 ppm)

Gene ontology analysis identified pathways related to:

19 genes

Number	Gene symbol	Biological process	Direction of change in expression
1	AIFM3	Apoptosis	Down
2	ARHGEF37	GTPase Activity	Up
3	BC051665	Proteolysis	Up
4	BTG4	Cell Cycle	Down
5	DZIP1L	Cilium Assembly	Down
6	FAM19A4	Membrane Potential	Up
7	FOS	Cellular Processes	Up
8	FOSB	Cellular Processes	Up
9	HPX	Iron Ion Homeostasis	Down
10	JDP2	Transcription	Down
11	MAPK3	MAPK Signaling	Down
12	MEF2D	Cell Signaling/Transcription	Down
13	NPAS4	Cellular Response to Stimuli/Transcription	Up
14	PTEN	Cellular Function	Down
15	PTPRC	Cellular Processes	Up
16	SOCS3	Cytokine Signaling	Up
17	SPRY1	Neurotrophin/TRK Signaling	Down
18	TGM1	Cellular Processes	Down
19	TMEM216	Cellular Organization	Up

Gene enrichment table of physiological system development and function for 72 hpf zebrafish larvae in all three groups

Gene alterations associated with

Physiological system development and function	p-Value[a]	Number of genes[b]
Hematological system development and function	7.14×10^{-3}–1.06×10^{-6}	7
Quantity of T lymphocytes	1.06×10^{-6}	7
Development of lymphocytes	3.03×10^{-4}	5
Differentiation of blood cells	1.64×10^{-3}	5
Proliferation of T lymphocytes	1.99×10^{-3}	6
Tissue morphology	6.25×10^{-3}–1.06×10^{-6}	8
Quantity of T lymphocytes	1.06×10^{-6}	7
Quantity of B lymphocytes	3.83×10^{-4}	4
Quantity of oligodendrocytes	4.43×10^{-4}	2
Tissue development	7.14×10^{-3}–3.09×10^{-6}	10
Proliferation of smooth muscle cells	1.82×10^{-4}	4
Proliferation of fibroblasts	2.49×10^{-5}	5
Differentiation of bone cells	3.09×10^{-6}	6
Growth of connective tissue	5.83×10^{-5}	6
Connective tissue development and function	7.14×10^{-3}–4.41×10^{-6}	10
Proliferation of fibroblast cells	2.49×10^{-5}	5
Formation of osteoclasts	4.21×10^{-6}	4
Differentiation of osteoclasts	3.34×10^{-5}	4
Embryonic development	7.14×10^{-3}–4.41×10^{-6}	10
Development of body trunk	1.23×10^{-4}	7
Formation of osteoclasts	4.21×10^{-6}	4
Development of lymphatic system	4.99×10^{-4}	4
Development of abdomen	3.55×10^{-3}	4

Caballero-Gallardo et al., 2018

Gene enrichment table of diseases and disorders in 72 hpf zebrafish larvae in all three aqueous coal dust treatments

Diseases and disorders	*p*-Value[a]	Number of genes[b]
Connective tissue disorders	1.34×10^{-3}–1.00×10^{-6}	7
Arthritis	5.50×10^{-4}	6
Rheumatoid arthritis	9.23×10^{-4}	5
Osteoporosis	1.34×10^{-4}	2
Immunological disease	7.14×10^{-3}–1.00×10^{-6}	7
Rheumatoid arthritis	9.23×10^{-4}	5
Splenomegaly	1.15×10^{-6}	4
Thymic lymphoma	1.34×10^{-3}	2
Inflammatory disease	7.14×10^{-3}–1.00×10^{-6}	8
Chronic inflammatory disorder	5.55×10^{-4}	6
Airway hyperresponsiveness	1.33×10^{-4}	3
Inflammation of the liver	2.93×10^{-3}	3
Skeletal and muscular disorders	6.25×10^{-3}–1.00×10^{-6}	8
Arthritis	5.50×10^{-4}	6
Rheumatoid arthritis	9.23×10^{-4}	5
Arthritis of the ankle joint	1.59×10^{-5}	2
Cancer	7.14×10^{-3}–5.38×10^{-6}	10
Hyperplasia	2.53×10^{-4}	5
Prostate cancer and tumors	9.23×10^{-4}	5
Bladder cancer	2.32×10^{-3}	4
Development of tumor	3.94×10^{-4}	4

Altered genes were associated with diseases and disorders

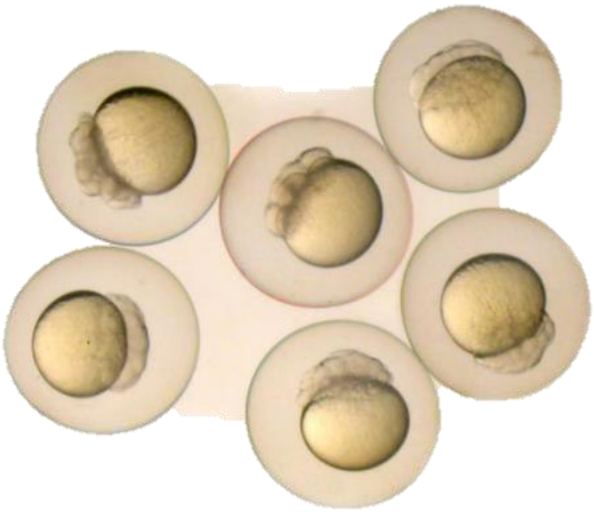
a Derived from the likelihood of observing the degree of enrichment in a gene set of a given size by chance alone. b Classified as being differentially expressed that relate to the specified function category; a gene may be present in more than one category.

Caballero-Gallardo et al., 2018

Gene enrichment table of molecular and cellular function in 72 hpf zebrafish larvae in all three aqueous coal dust treatments

Analysis of the 19 genes altered in all three groups showed that they were associated with:

Molecular and cellular function	*p*-Value[a]	Number of genes[b]
Cellular development	7.14×10^{-3}–3.09×10^{-6}	13
Differentiation of cells	8.15×10^{-6}	11
Differentiation of connective tissue	8.23×10^{-6}	7
Differentiation of bone cells	3.09×10^{-6}	6
Differentiation of blood cells	1.64×10^{-3}	5
Cellular growth and proliferation	7.14×10^{-3}–4.21×10^{-6}	11
Proliferation of cells	1.21×10^{-3}	11
Formation of cells	6.95×10^{-4}	6
Proliferation of fibroblasts	2.49×10^{-5}	5
Cell cycle	6.25×10^{-3}–7.57×10^{-6}	7
Cell cycle progression	1.07×10^{-4}	7
Mitosis	1.85×10^{-3}	4
Senescence of cells	1.55×10^{-3}	3
Cell death and survival	7.14×10^{-3}–7.57×10^{-6}	12
Necrosis	2.72×10^{-4}	10
Apoptosis	3.51×10^{-4}	10
Cell survival	8.91×10^{-5}	8
Cell viability	2.68×10^{-3}	6
Gene expression	5.36×10^{-3}–1.52×10^{-5}	9
Transcription of RNA	7.80×10^{-4}	8
Binding of DNA	1.52×10^{-5}	6
Transactivation of RNA	2.85×10^{-4}	5

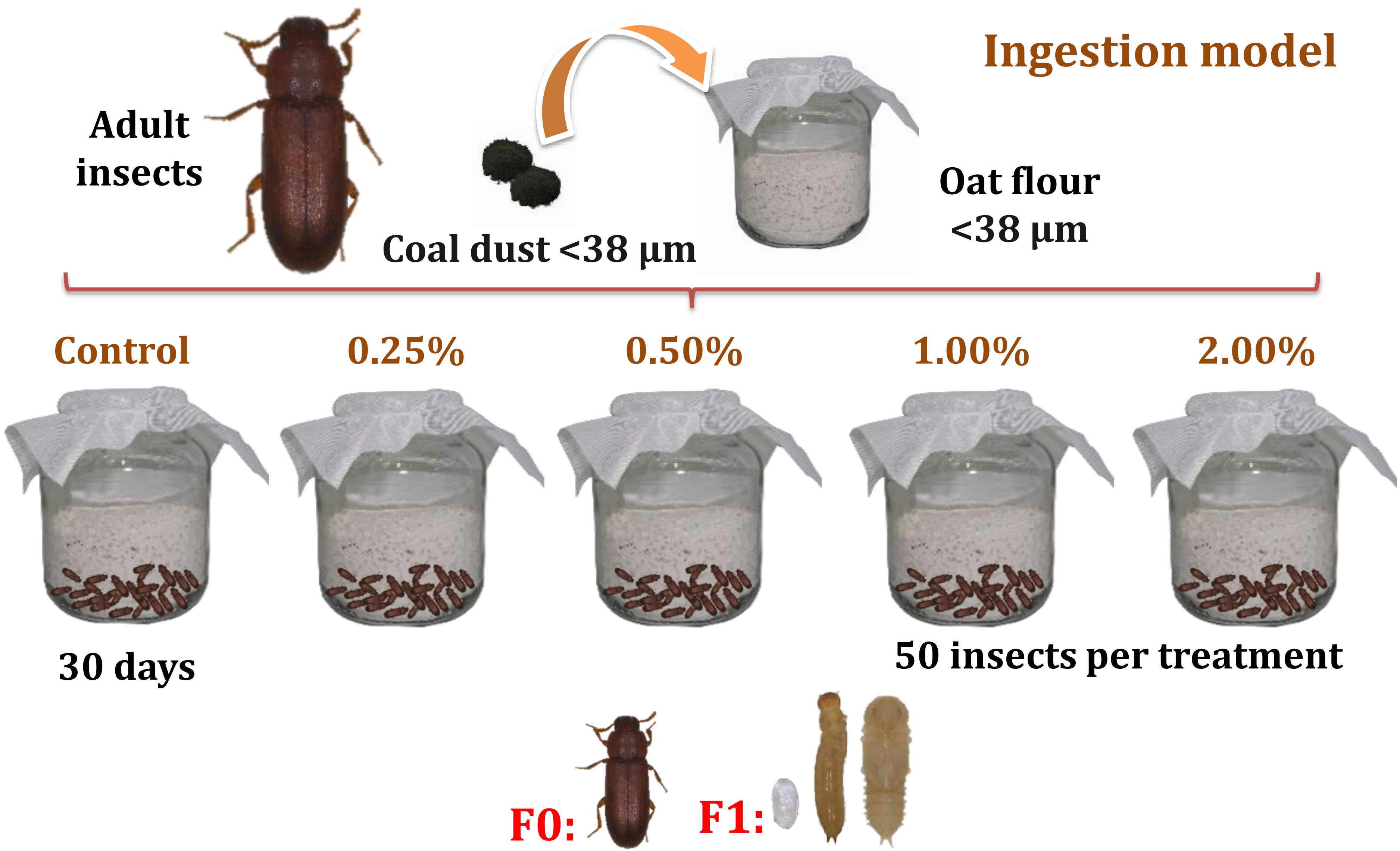


This was the first study to characterize gene expression changes in response to an aqueous coal dust extract

Although no morphological alterations were observed, transcriptome results suggested systems to target in future researches

Caballero-Gallardo et al., 2018

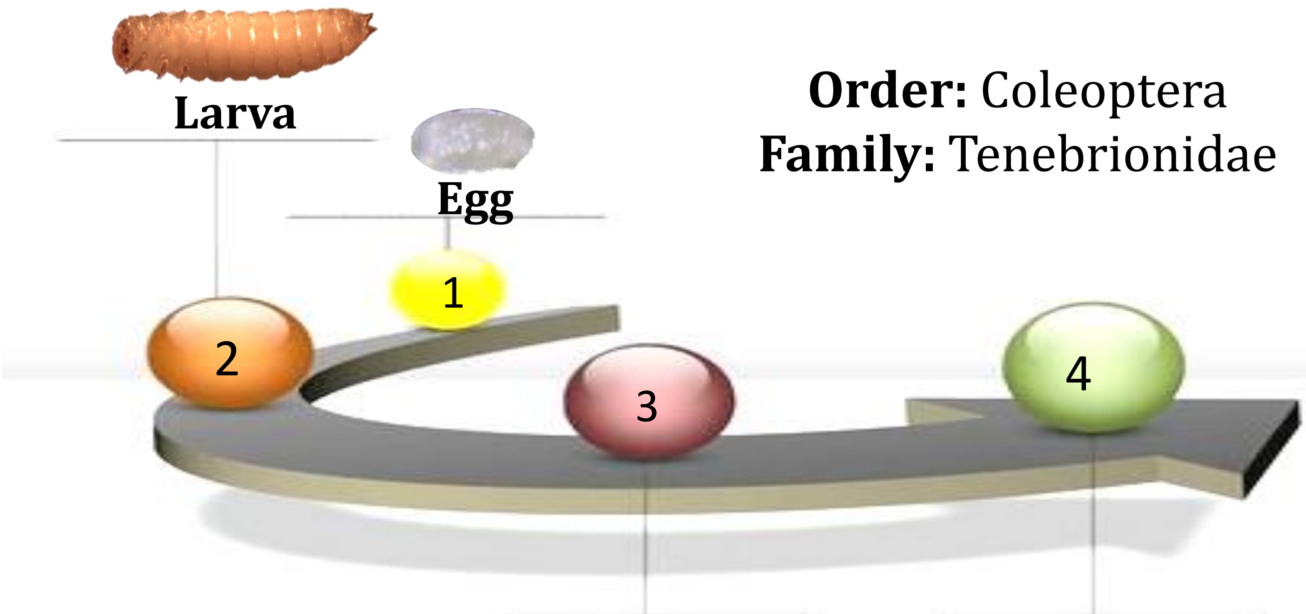
The red flour insect *Tribolium castaneum*, Herbst



Effects on Mortality and progeny

Tribolium castaneum an alternative model for the study of toxicological

Order: Coleoptera
Family: Tenebrionidae



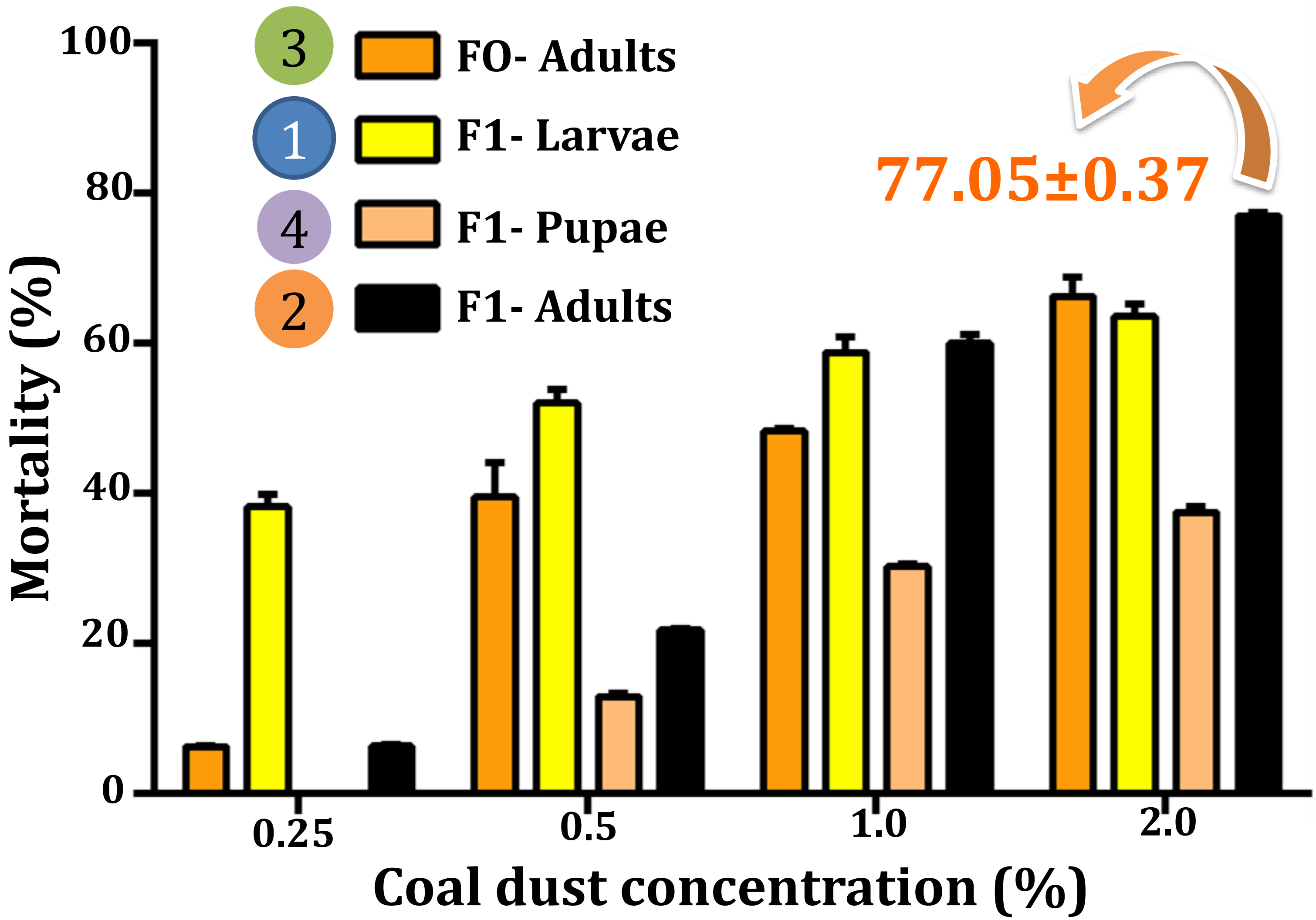
Pupa

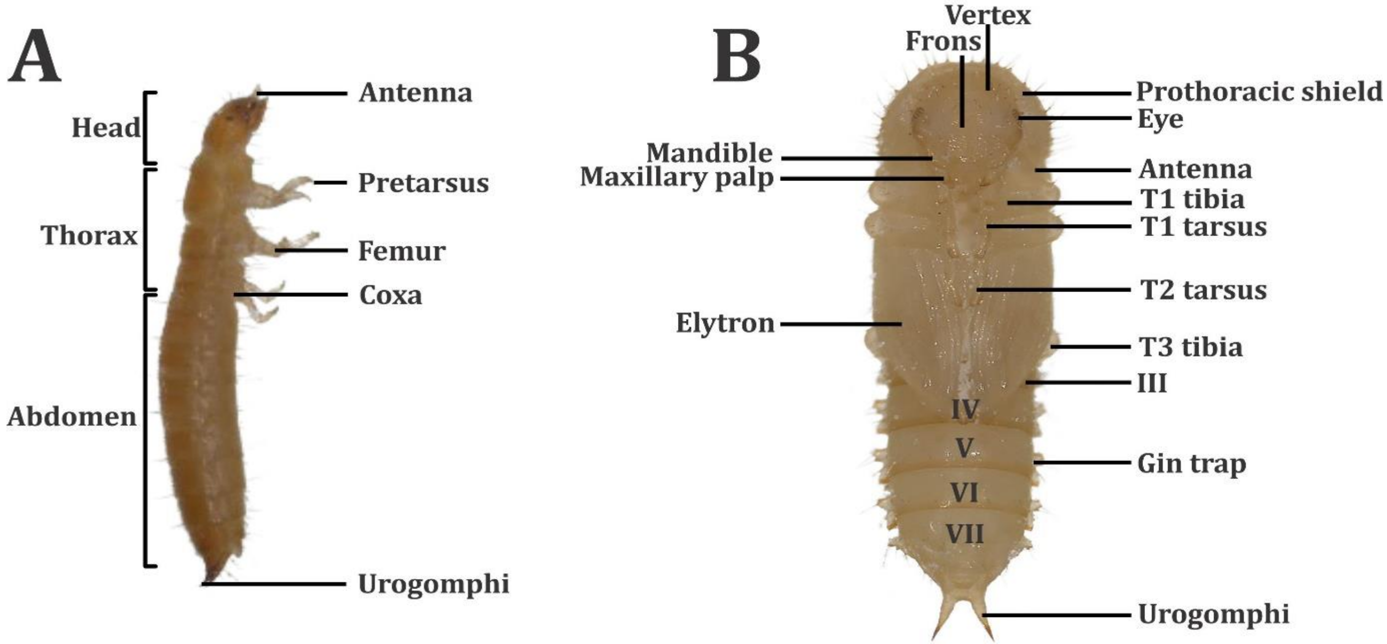
Different stages of the adult

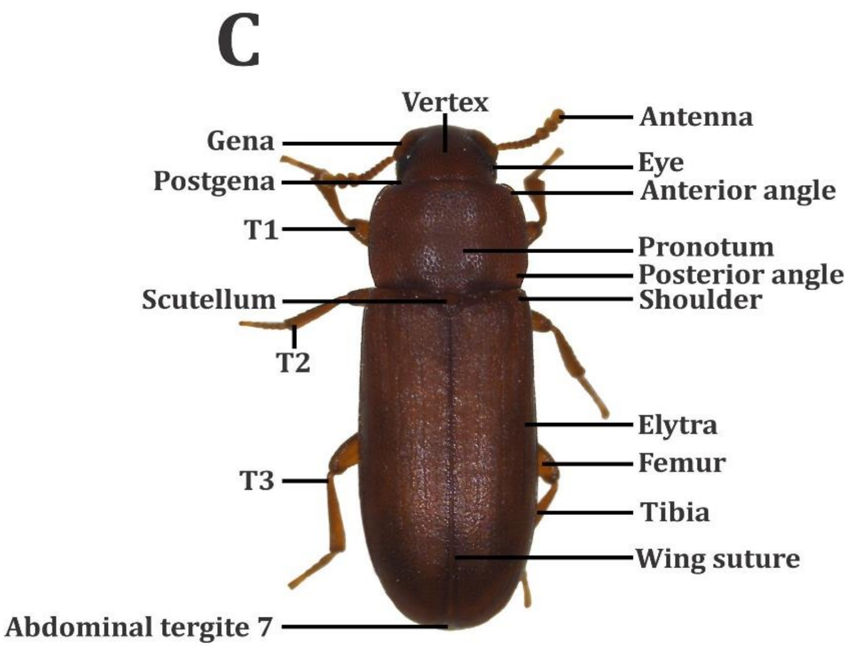
Rapid life cycle
Very economical
Easy cultivation
Its genome is sequenced

FAO, 2009, 2010; Olivero-Verbel et al., 2009

Percentages of mortality found for different life stages of *T. castaneum* from two generations, after 30 days of exposure





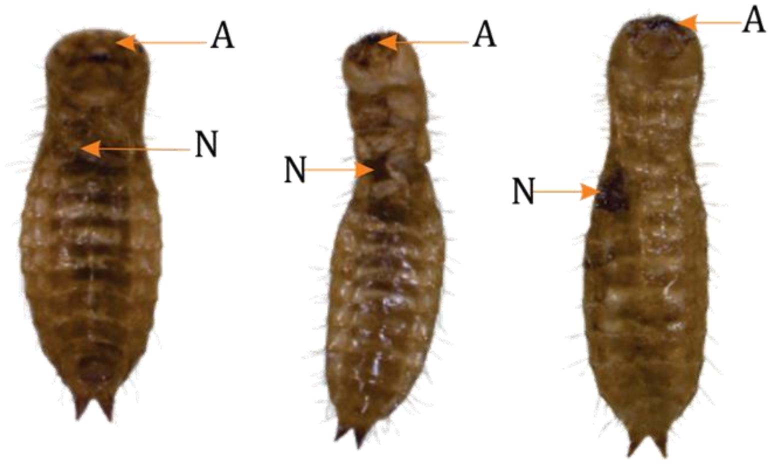


Morphological structures of Larvae (A), Pupae (B) and Adults (C) of the *Tribolium castaneum* species.

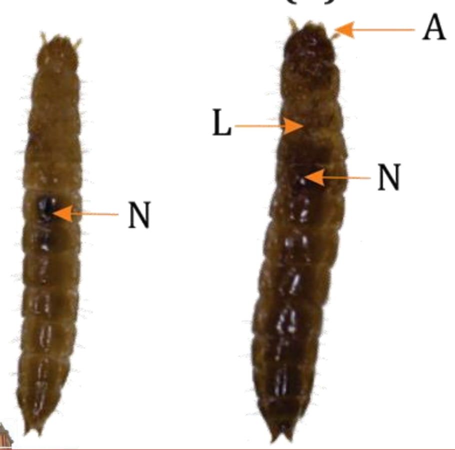
Morphological abnormalities on the development of the insect progeny after exposure to coal dust in flour

Larvae with thorax size reduction

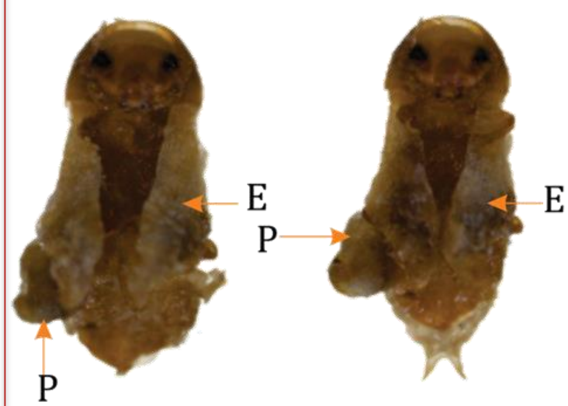
Larvae with absence of antennas (A) and necrosis (N)



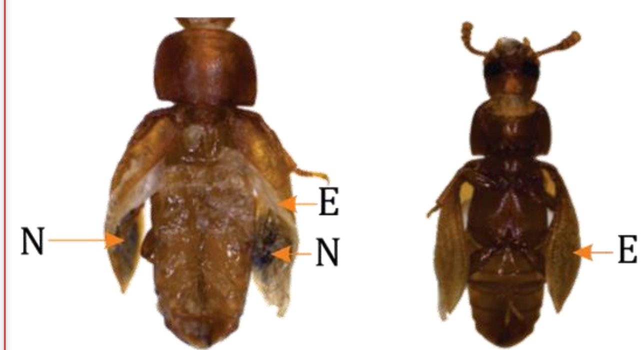
Larvae with lack of legs (L) and necrosis (N)



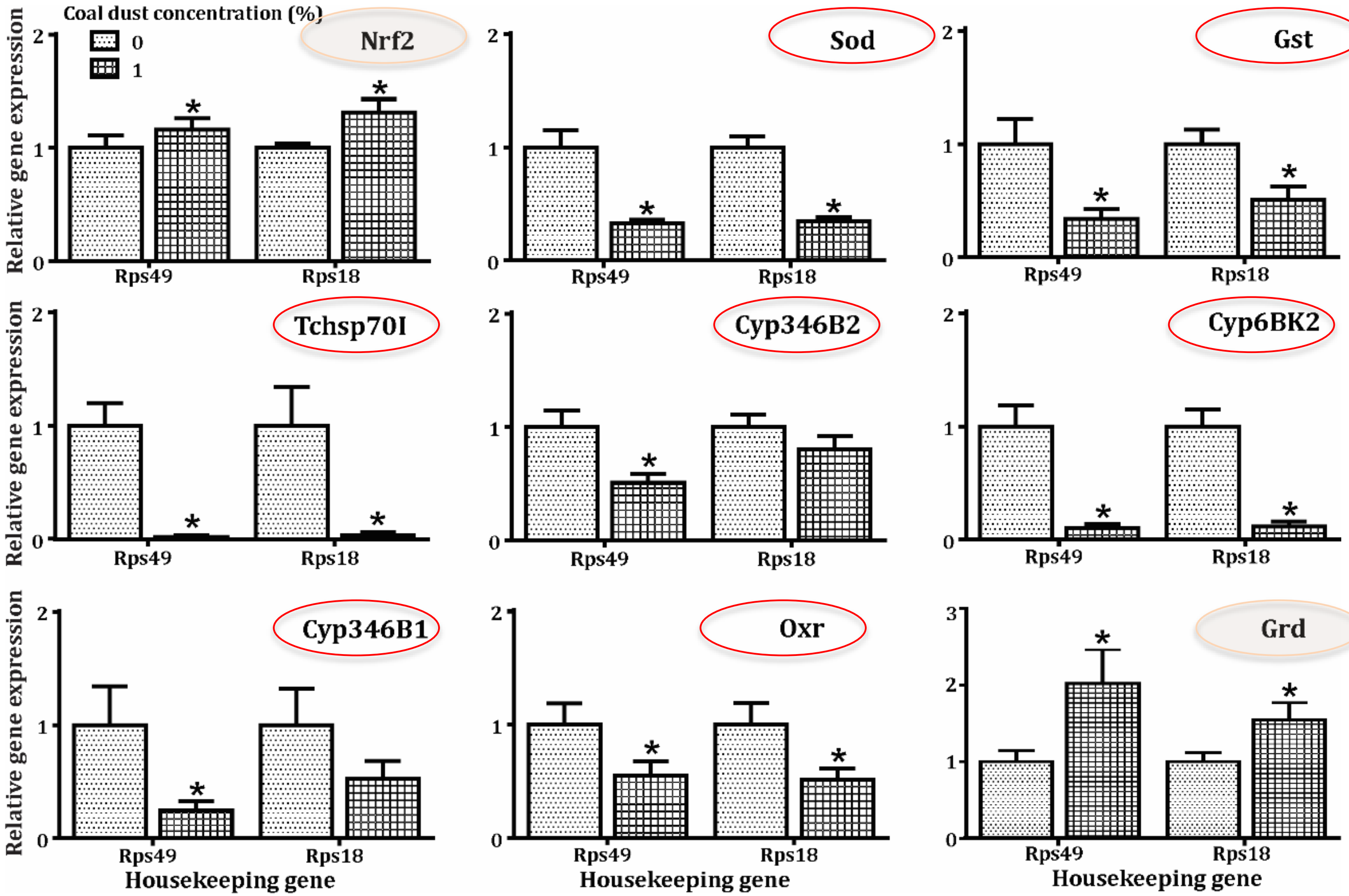
Pupae with protuberance (P) and malformed elytron (E)



Adults with malformed elytra (E) and necrosis (N)



Relative expression of genes related to oxidative stress, thermal shock, metabolism, and synaptic transmission in adult *T. castaneum* after exposure to coal dust



Expression was normalized against Rps49 and Rps18 (housekeeping genes). * Significant differences ($p<0.05$) when compared to the control. Nrf2: Nuclear factor erythroid 2–related factor 2.

Remarks

- Parental exposure implies several morphological, genetic and survival responses on progeny, which highlights the great toxicological potential that this air pollutant has.

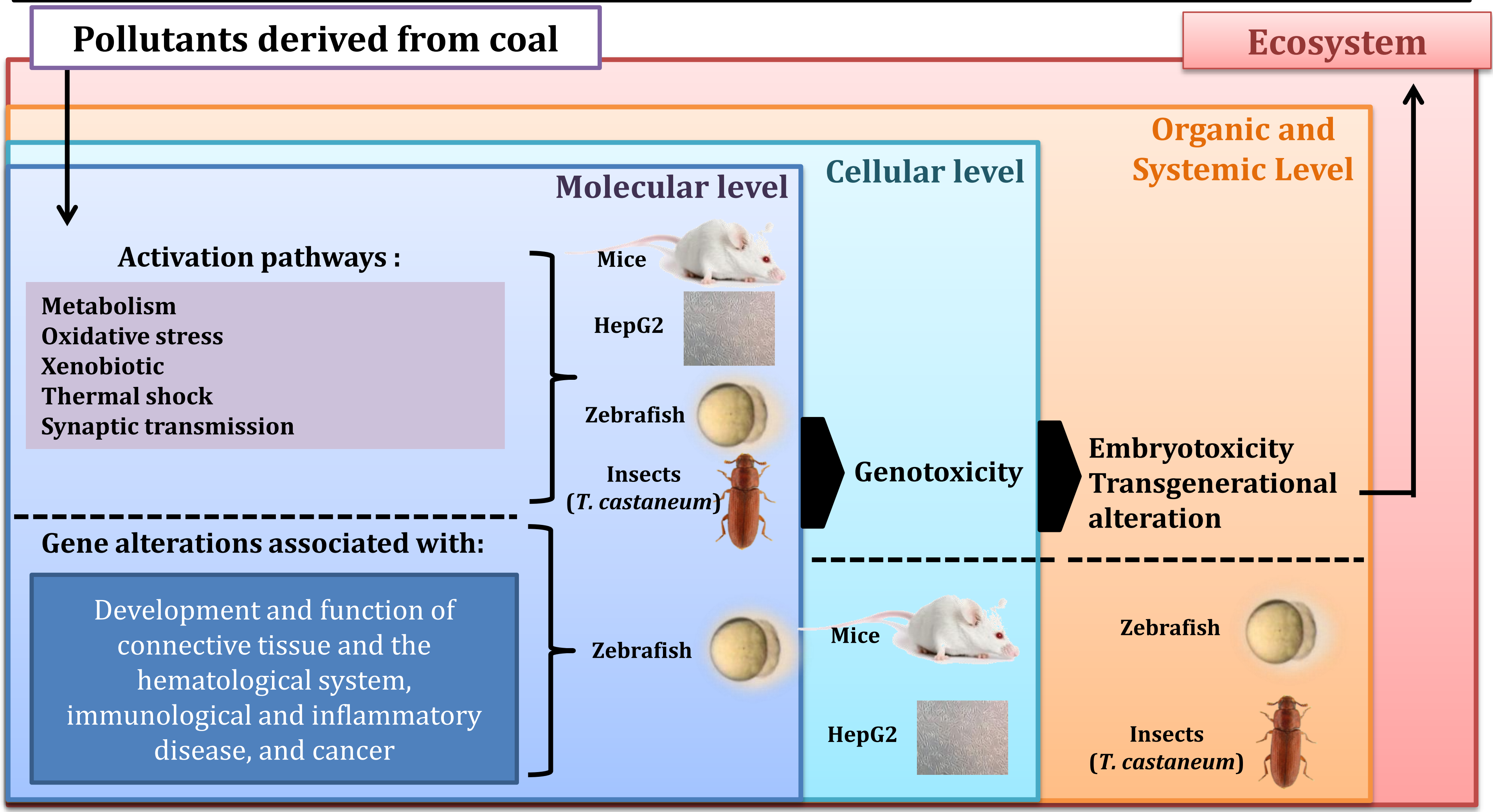
- A number of complex effects at molecular and morphological level were observed when the biological model was fed on coal dust-contaminated diet.

- High percentages of mortality were found at all life stages as a result of the concentration used.

- We consequently propose *T. castaneum* as an alternative organism to evaluate the potential toxicological effects on following generations associated with the exposure to coal dust in high-polluted environments, as is the case of the areas in which mining activities are carried out.

Caballero-Gallardo et al., 2018

The shipment of coal in Colombian ports has a negative impact on marine sediments. These effects are measurable in cellular models, suggesting these residues aren't inert at all and interact with biota.
Aqueous coal extracts, similarly as sediment particles do, are active in a fish toxicity model, producing genomic alterations.
Finally, exposure to coal dust can generate a series of toxicological effects at molecular, cellular and physiological levels, as well as in the progeny.



рахмат
danke
謝謝
ngiyabonga
spas
teşekkür ederim
Баярлалаа
mersi
kia ora
barka
welalin
tack
mahalo
tapadh leat
спасибо
faafetai lava
vinaka
спасибі
blagodaram
dank je
misaotra
matondo
paldies
grazzi
kiitos
dankie
nanni
nandri
dhanyavad
mauruuru
thank you
gracias
хвала
asante
manana
bedankt
bayarlalaa
gràçie
hvala
köszönöm
dankon
aciū
akun
obrigada
murakoze
tenki
enkosi
djiere dieuf
tau
mochchakkeram
dziękuję
дякую
mamnun
chnorakaloutioun
gratias ago
gràcies
sulpáy
go raibh maith agat
chokrane
sobodi
dėkuji
sagolun
sukriya
kop khun krap
taiku
arigatô
takk
dakujem
trugarez
obrigado
mèsi
didi madloba
najis tuke
ありがとう
tanemirt
grazie
shukriya
mercé
мерси
kam sah hamnida
rahmat
terima kasih
rahmet
ευχαριστώ
diolch
dhanyavadagalu
merci
তোমাকে ধন্যবাদ
감사합니다
xièxie

Acknowledgements

University of Cartagena
Environmental and Computational Chemistry Group
The Colombian Academy of Exact, Physical and Natural Sciences
Colombian Network of Women Scientists
IANAS Women for Science Program
Brazilian Academy of Sciences

kcaballerog@unicartagena.edu.co