Complex Mixture-associated Hormesis and Toxicity: The Case of Leather Tanning Industry

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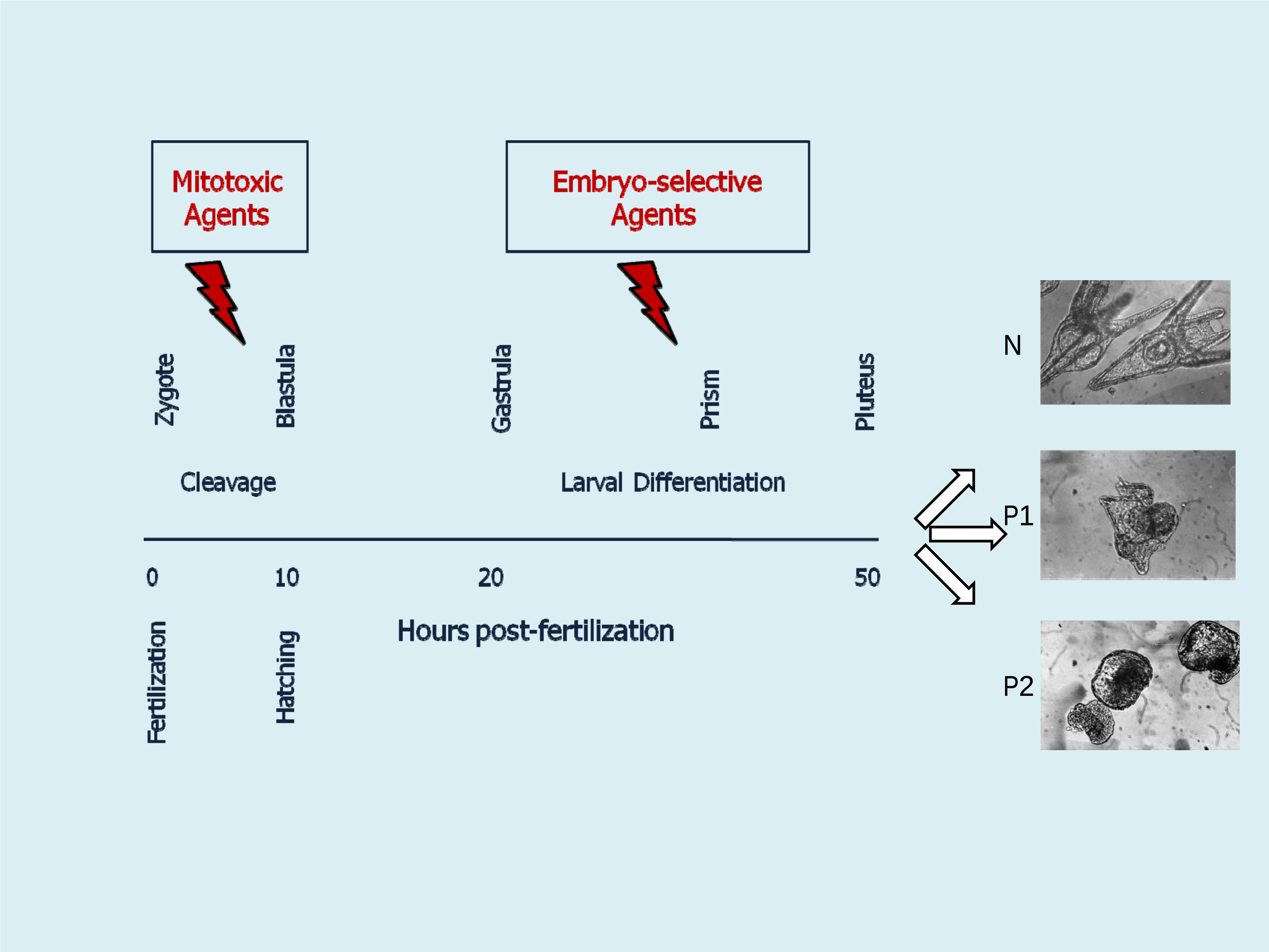
Testing Complex Mixtures

Toxicological and genotoxicological investigation of complex mixtures is one of the main focus of the recent research in toxicology. **Testing complex mixtures presents a formidable scientific problem since most recently available toxicological data have been obtained from single substance studies and are not simply transferable to mixtures of chemicals.**

Groten *et al.* 2001
Trends Pharmacol Sci 22:316-22

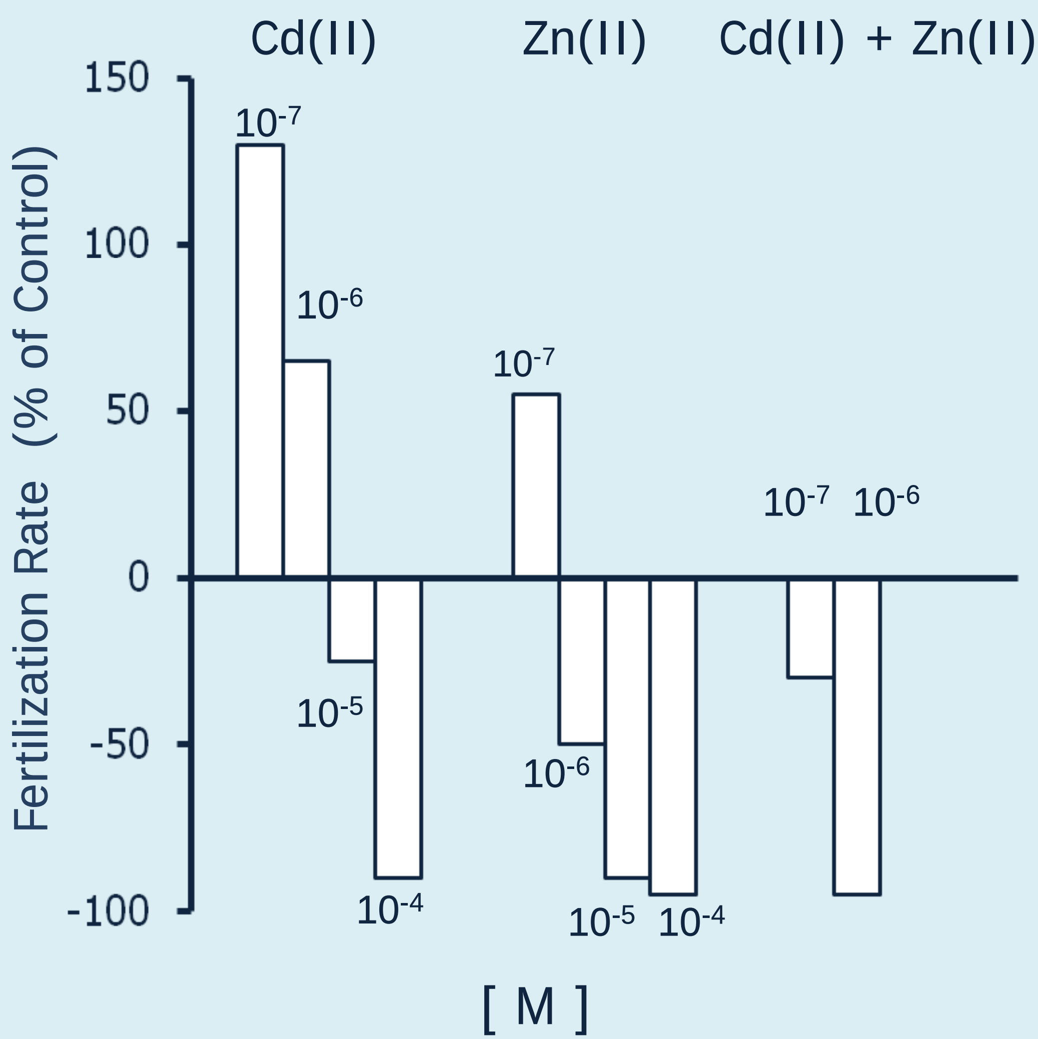
Main laboratory procedures and endpoints in sea urchins embryo and sperm bioassays

Testing object	Duration	Endpoints
Embryos (zygote → pluteus)	up to 72 hrs	1) larval malformations 2) developmental arrest 3) embryonic mortality 4) cytogenetic aberrations
Sperm	1 hr	1) fertilization rate 2) offspring quality


Mitotoxic Agents
Embryo-selective Agents
Zygote
Blastula
Gastrula
Prism
Pluteus
Cleavage
Larval Differentiation
0
10
20
50
Fertilization
Hatching
Hours post-fertilization
N
P1
P2

Published literature from sea urchin embryo tests
(from MedLine and Toxline, April 2008, and author’s archives)

Classes of agents	No. agents
Inorganics	12
Pharmaceuticals	28
Environmental/occupational agents	21
Natural products	7
Miscellaneous	9



Pagano et al. 1986

STUDY DESIGN IN EVALUATING HORMETIC EFFECTS

The evaluation of concentration-related shifts from hormesis to toxicity requires adequate design in bioassays, including:

a) broadly ranging agent concentrations, not confined to NOAEL;

b) adequate definition of controls.

Defining control quality criteria in toxicity bioassays

- control quality is commonly assumed to be "optimal" (zero frequency of adverse events);
- by definition, "100% normal" controls do not permit any observation of hormetic effects;
- controls are required to be characterized by low (sub-optimal) culture quality;
- control quality was re-defined by accepting controls with >30% developmental defects.

De Nicola et al. 2006

RE-DEFINING ACCEPTANCE CRITERIA FOR CONTROLS

Hormetic effects in sea urchin bioassays have been reported as changes in fertilization success, by maintaining fertilization rate (FR) in controls at suboptimal levels, i.e. 50 to 70% (Pagano et al., 1986; De Nicola et al., 2004).

In order to evaluate any hormetic effects also in the embryotoxicity bioassays, the acceptance criteria for control cultures were re-defined, by assuming that “low-quality” control cultures (assigned as having <70% viable pluteus larvae) provide information allowing us to discern both toxic and hormetic responses in terms of either developmental toxicity or amelioration of larval quality or viability.

SOME REPORTS ON HORMESIS FROM OUR TEAM

Pagano G, Esposito A and Giordano GG. (1982) Fertilization and larval development in sea urchins following exposure of gametes and embryos to cadmium. *Arch Environ Contam Toxicol* **11**:47-55.

Pagano G, Esposito A, Bove P et al. (1982) Arsenic-induced developmental defects and mitotic abnormalities in sea urchin development. *Mutat Res* **104**:351-354.

Pagano G, Cipollaro M, Corsale G et al. The sea urchin: Bioassay for the assessment of damage from environmental contaminants. In: Cairns, J, Jr (Ed.) *Community Toxicity Testing*. Association for Standard Testing and Materials, Philadelphia, 1986, pp 67-92.

Pagano G, Iaccarino M, Guida M et al. (1996) Cadmium toxicity in spiked sediment to sea urchin embryos and sperm. *Mar Environ Res* **42**:54-55.

Korkina LG, Deeva IB, Iaccarino M, et al. (2000) Redox-dependent toxicity of diepoxybutane and mitomycin C in sea urchin embryogenesis. *Carcinogenesis* **21**:213-220.

Pagano G, Korkina LG, Iaccarino M et al. Developmental, cytogenetic and biochemical effects of spiked or environmentally polluted sediments in sea urchin bioassays. In: Garrigues, P, Walker, CH and Barth, H (Eds.) *Biomarkers in Marine Ecosystems: A Practical Approach*. Elsevier, Amsterdam, 2001, pp 85-129.

Pagano G, De Biase A, Deeva IB et al. (2001) The role of oxidative stress in developmental and reproductive toxicity of tamoxifen. *Life Sci* **68**:1735-1749.

De Nicola E, Gallo M, Iaccarino M et al. (2004) Hormetic vs. toxic effects of vegetable tannin in a multi-test study. *Arch Environ Contam Toxicol* **46**:336-344.

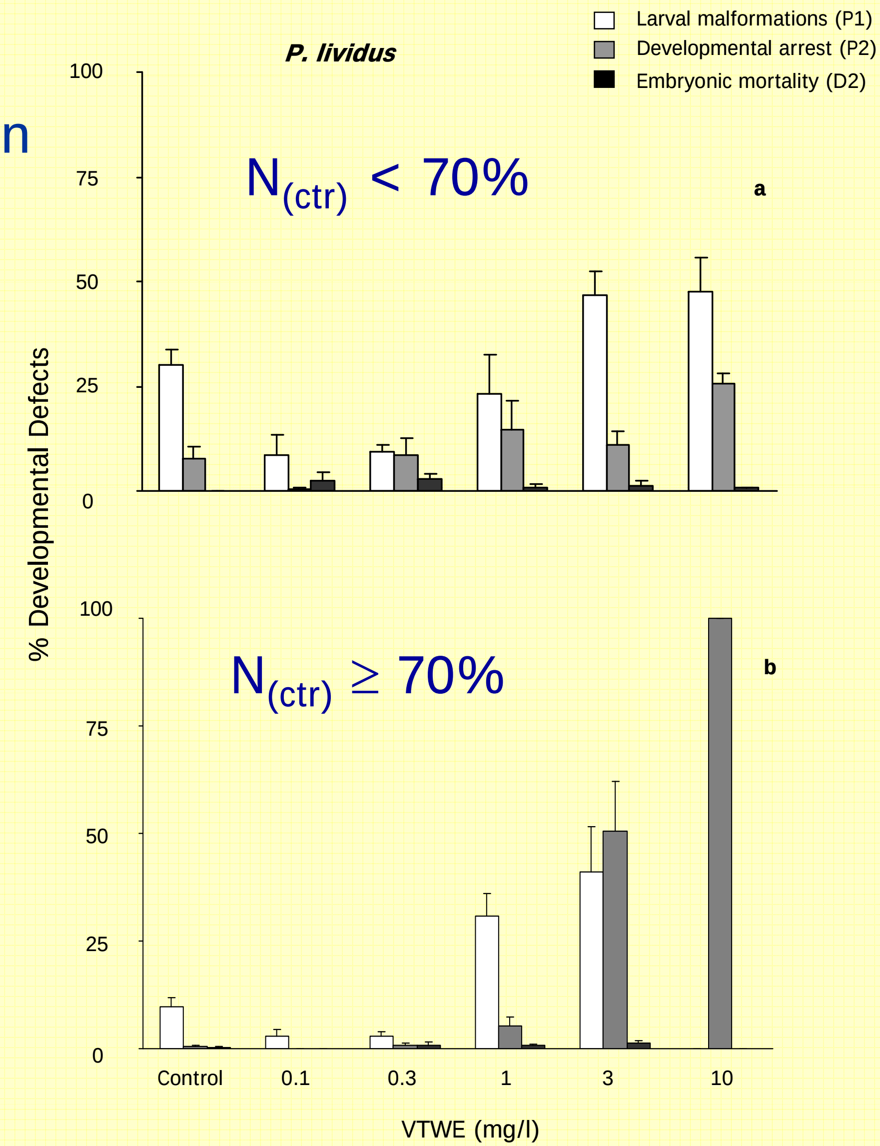
De Nicola E, Meriç S, Gallo M et al. (2007) Vegetable and synthetic tannins induce hormesis/toxicity effects in sea urchin early development and in algal growth. *Environ Poll* **146**:46-54.

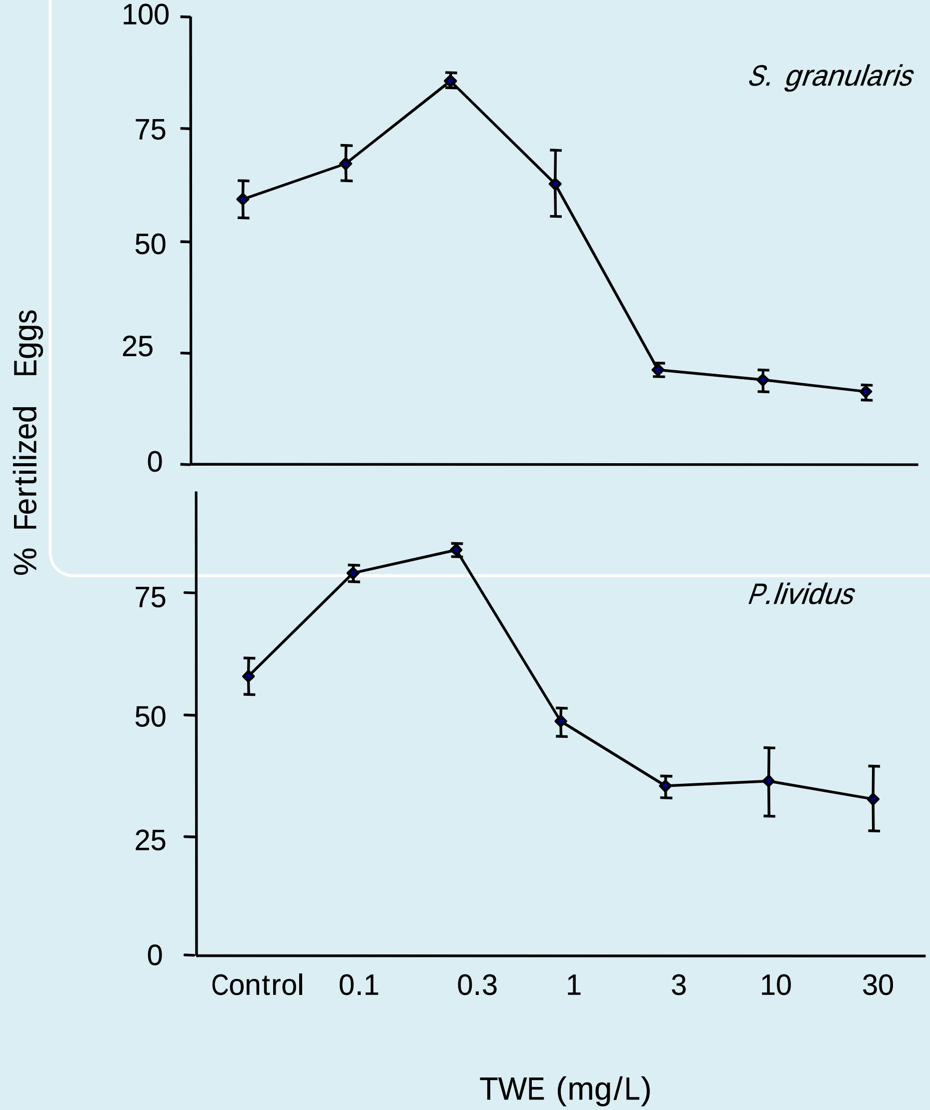
Selected information on tannin-related toxic and hormetic effects

Agents	Effects	References
Camellin B	induced apoptosis in HeLa cell line	Wang *et al.* (2001)
Hypericum perforatum extract & oil	↑ immunostimulating activity ↑ immunosuppressing activity	Anonymous (2001)
Gallic acid	non- toxic <5 g/kg body weight in mice	Rajalakshmik *et al*. (2001)
Areca nut polyphenols and tannin	oral cancer promotion	Jeng *et al*. (2001)
Terminalia arjuna tannin extract	↓ 2AF –induced mutagenicity	Kaur *et al.* (2000)
Tannic Acid	↑ metabolic activation of a few mutagens anticlastogenic and antimutagenic effects	Chen, Chung (2000) Sasaki *et al.* (1990)
Tannins	↑ inhibitory activity on lipid peroxidation	Hong *et al.* (1995)

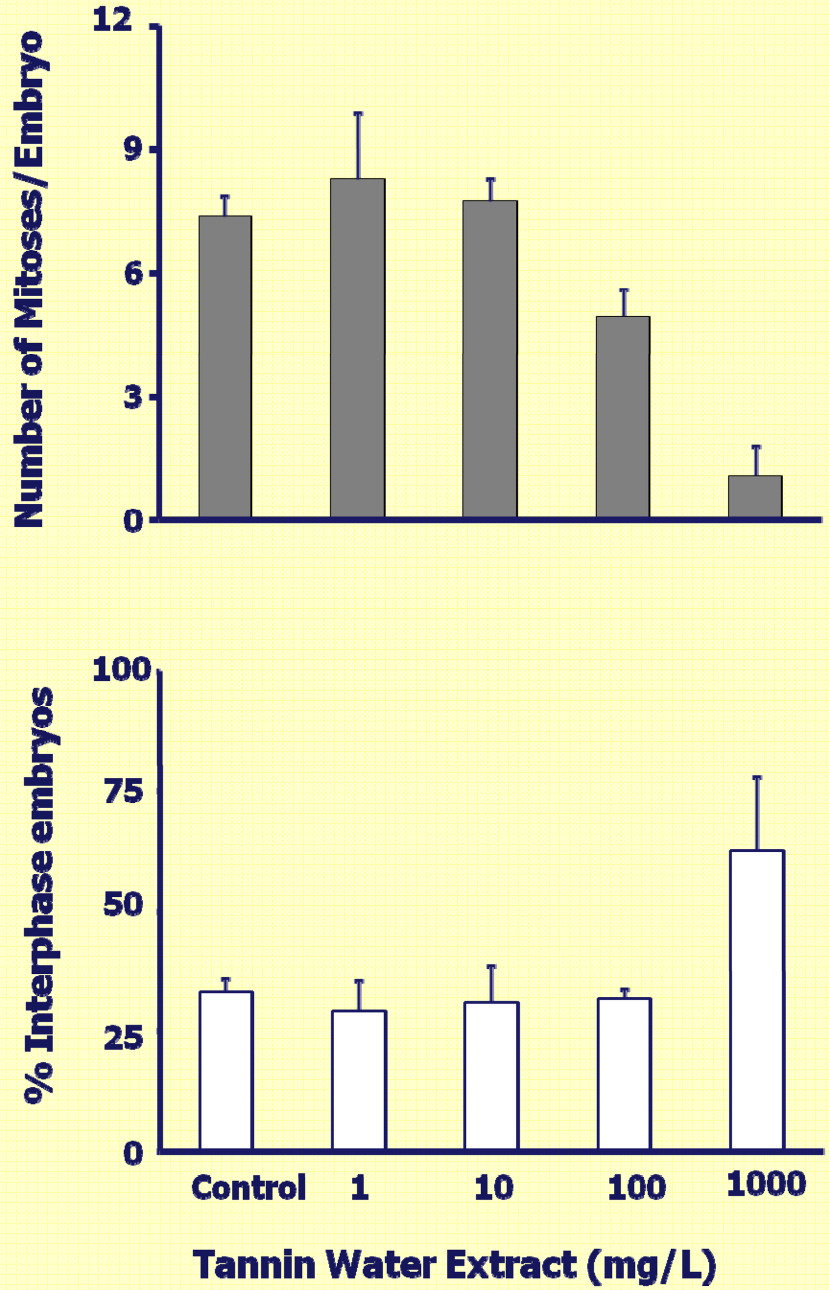
Vegetable tannin (*Acacia* sp.)

Embryo Exposure



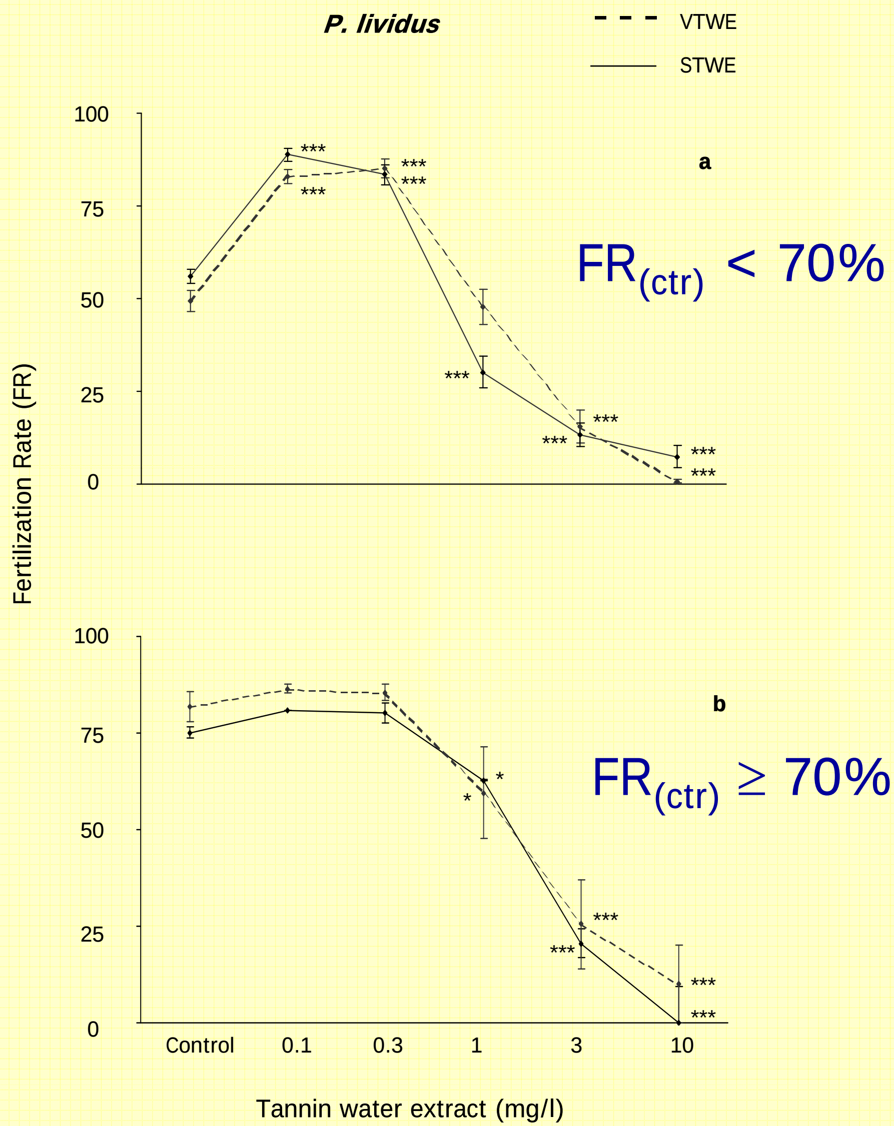


% Fertilized Eggs
100
75
50
25
0
S. granularis
75
50
25
0
P.lividus
Control
0.1
0.3
1
3
10
30
TWE (mg/L)

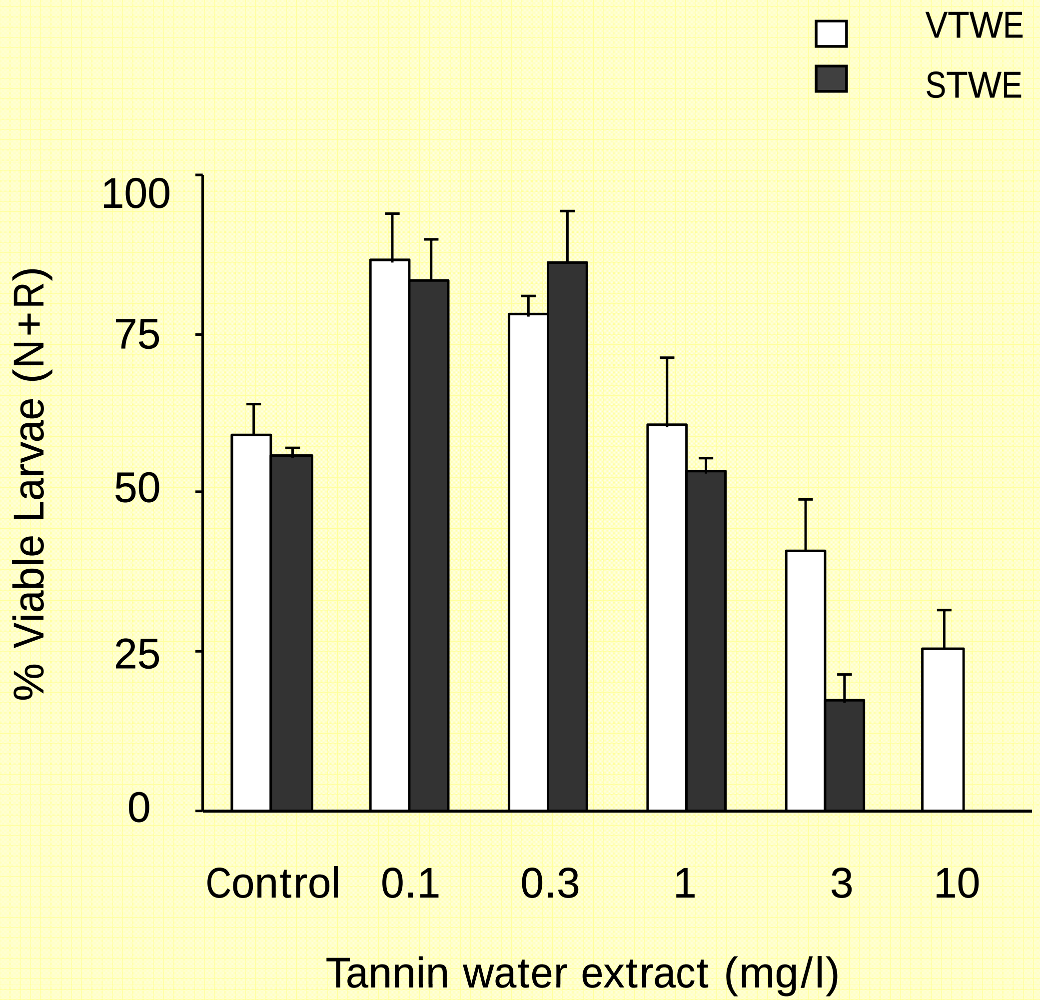

Number of Mitoses/Embryo
12
9
6
3
0
% Interphase embryos
100
75
50
25
0
Control
1
10
100
1000
Tannin Water Extract (mg/L)

Vegetable or Synthetic Tannin *(Acacia* sp.)

Sperm Exposure: Fertilization Success

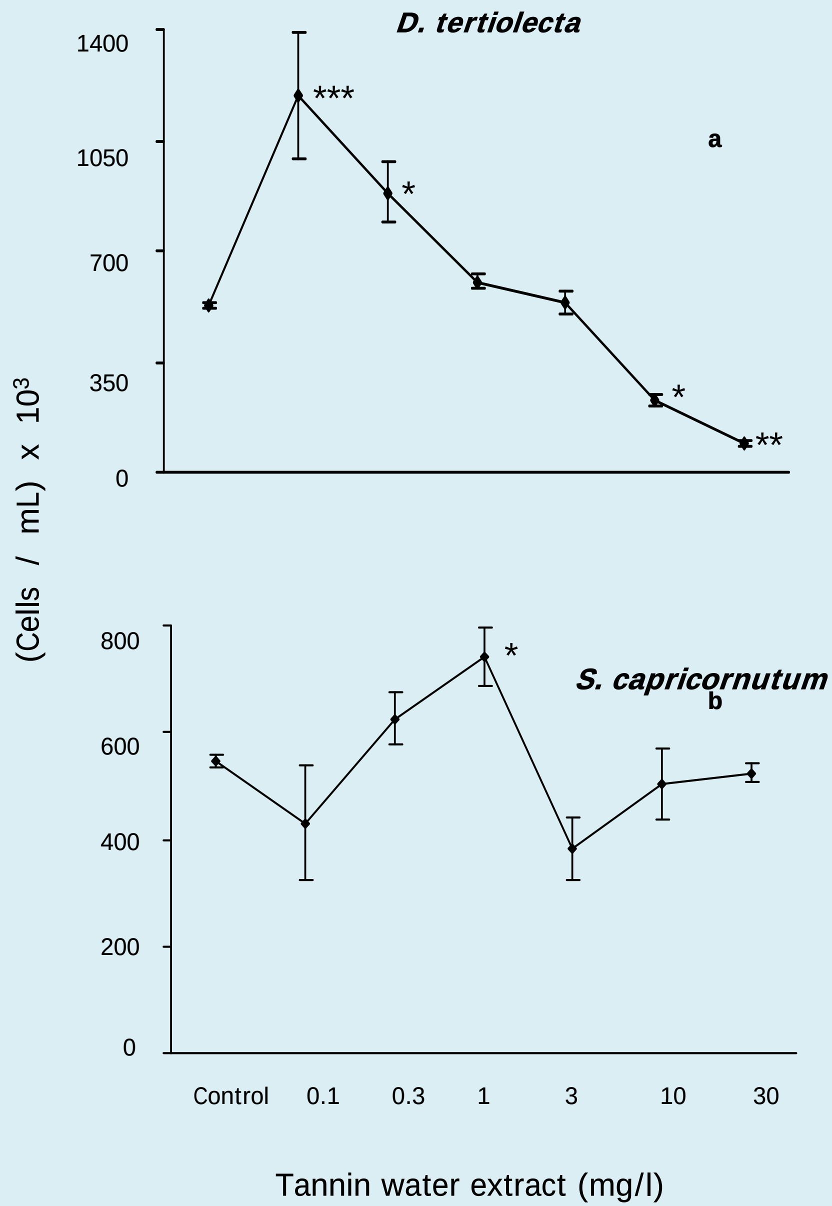


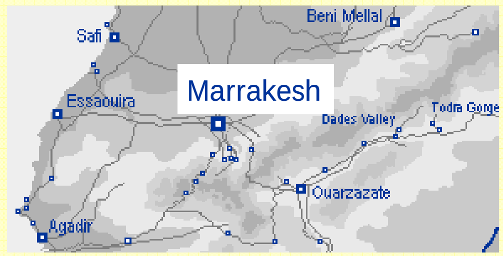
P. Lividus
Vegetable vs. synthetic tannins

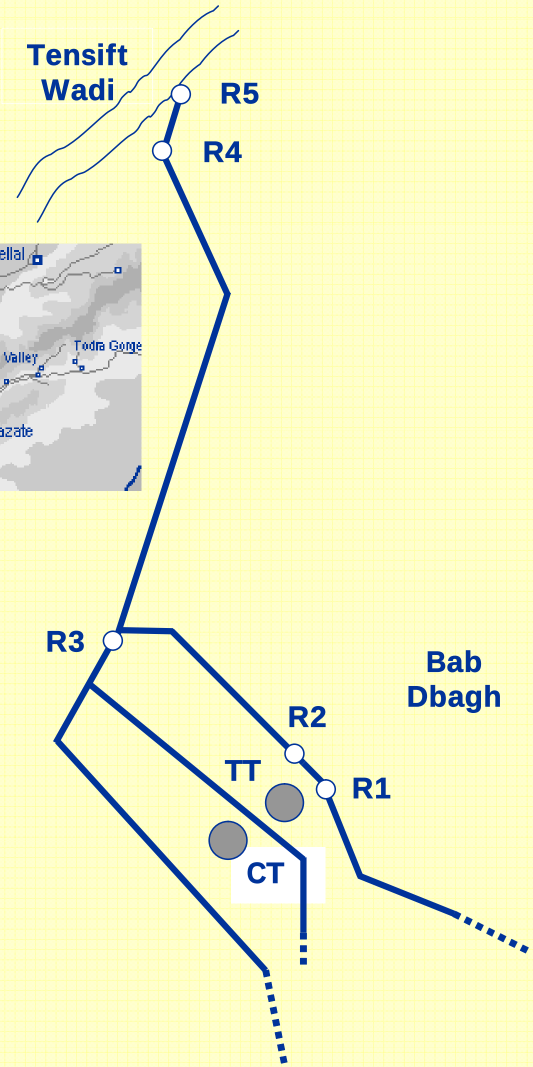


Dunaliella tertiolecta

Selenastrum capricornutum

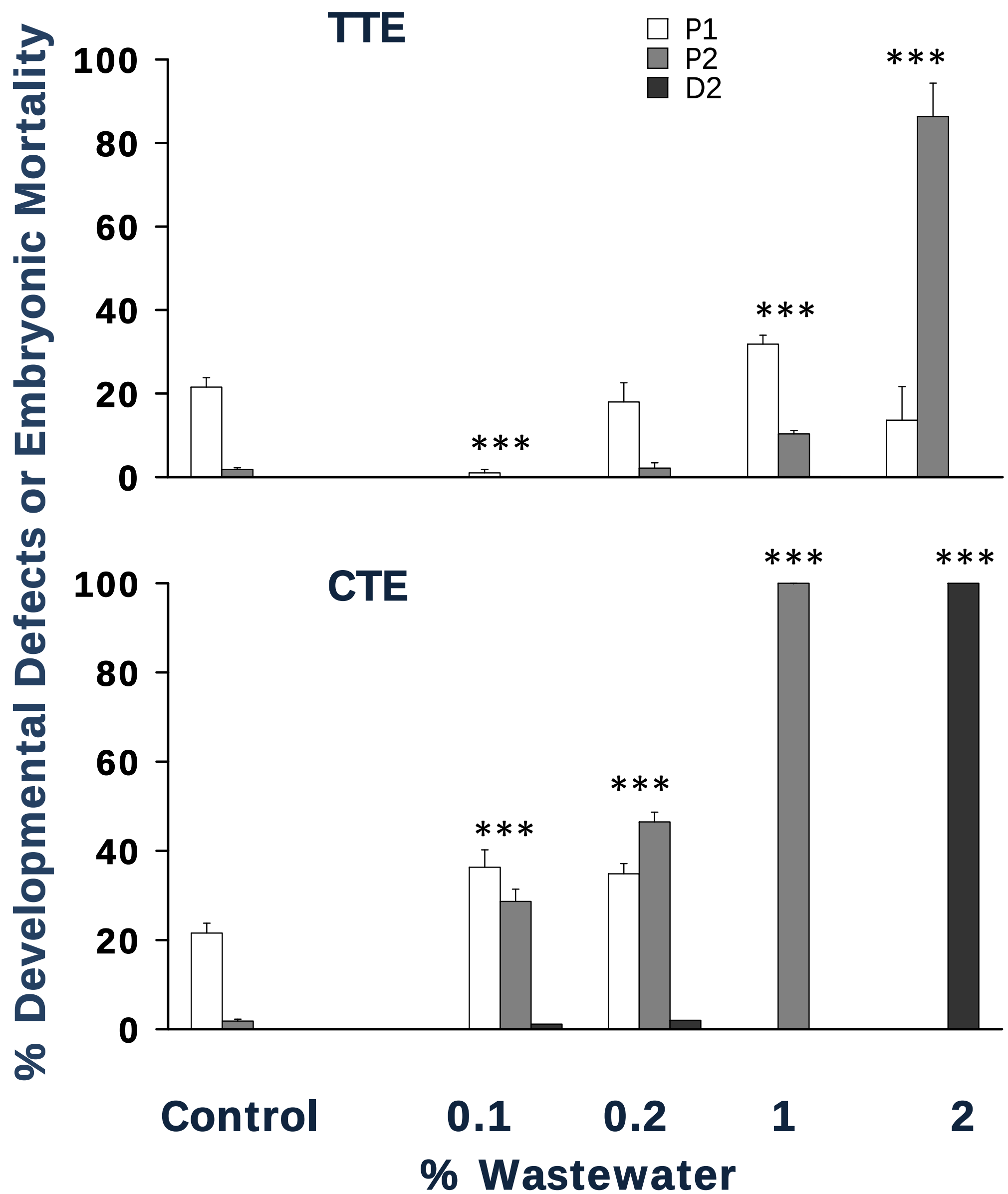






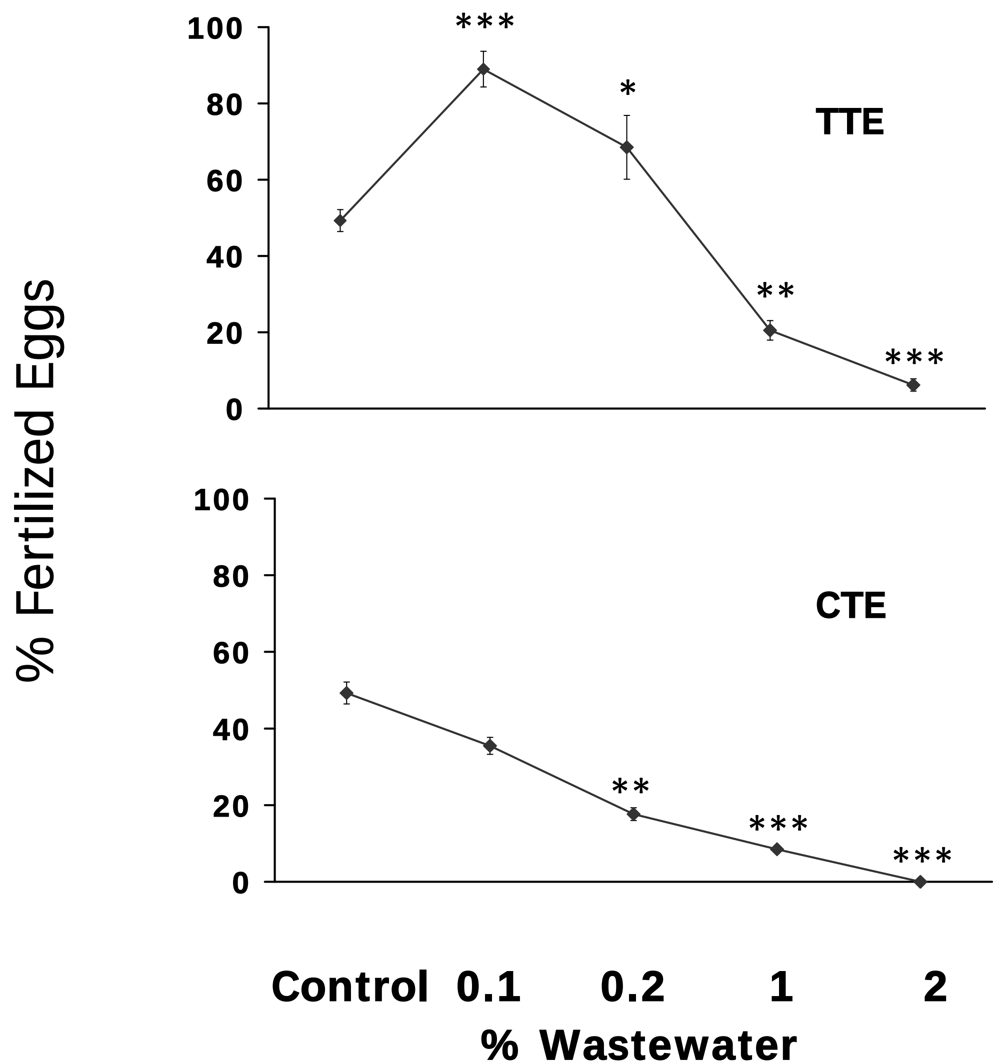
Tannery wastewater sewage
in Marrakesh, Morocco

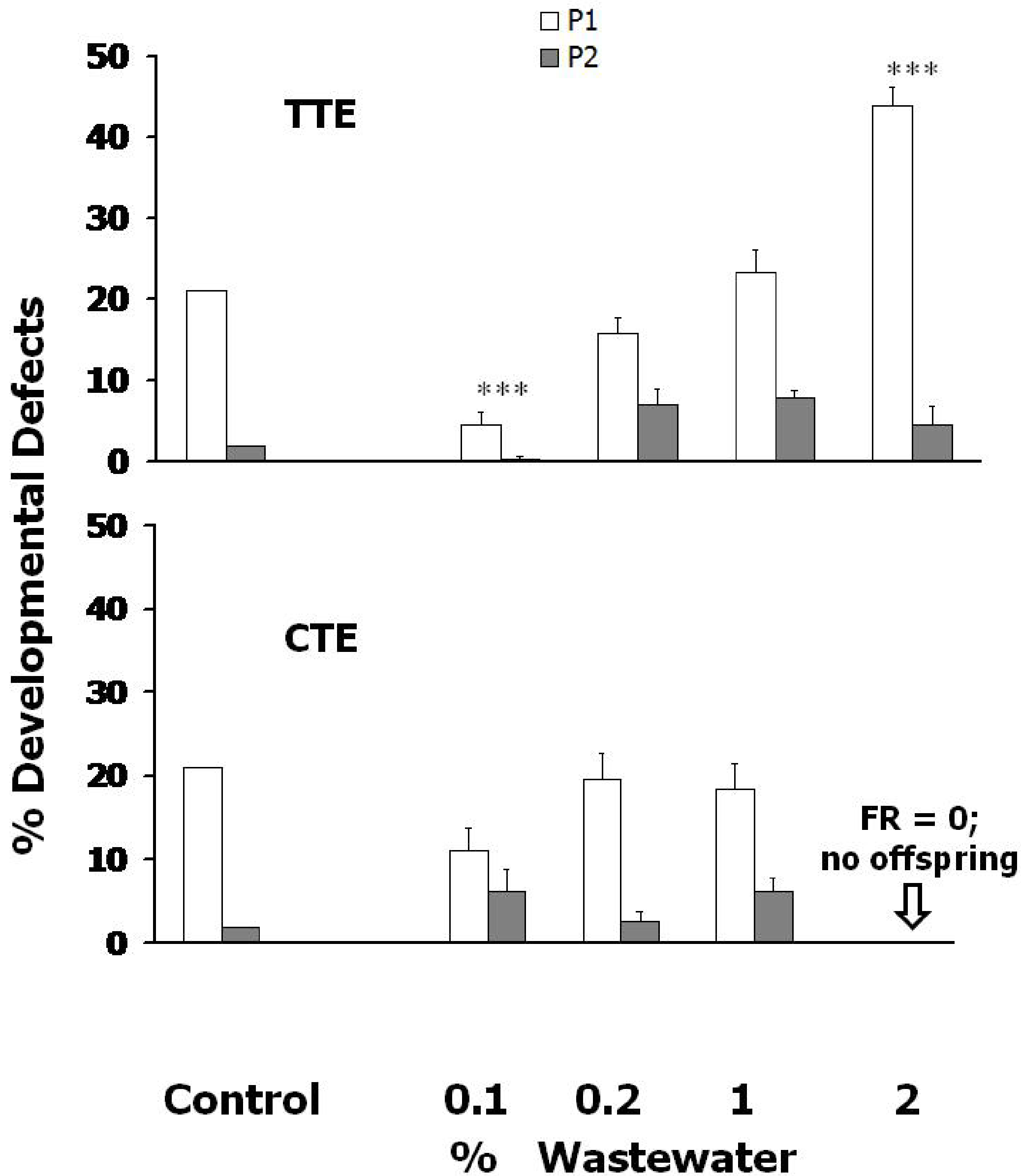
De Nicola et al. 2007



Tannin-based tannery effluent

Chromium-based tannery effluent


% Fertilized Eggs
100
80
60
40
20
0
***
*
**
***
TTE
100
80
60
40
20
0
CTE
**
***
***
Control
0.1
0.2
1
2
% Wastewater



P1
P2
TTE
CTE
% Developmental Defects
50
40
30
20
10
0
***
***
FR = 0;
no offspring
Control
0.1
0.2
1
2
% Wastewater

CONCLUSION

- Vegetable tannery effluent (TTE) results in lesser toxicity vs. chromium tannery effluent (CTE)

- Concentration-response trends are found:

  a) non-linear for TTE (hormesis/toxicity shift)

  b) monotonic for CTE (toxicity also at low levels)

- Prospect to renewing the interest to extending applications of vegetable tanning process

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