



المعهد العالي للبيوتكنولوجيا بالمنستير
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**Biochemical and proteomics responses
in Mediterranean crab: Effective tools
to assess the toxic effects of marine
contaminants**

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Intensification of anthropogenic activities on the coastal area



Urban and industrial discharges



Pollution and alteration of marine ecosystem quality



Endangers the Public and environmental health

**NEED TO
MONITOR THE
MARINE
ENVIRONMENT
contamination**



Strategy of Marine Ecosystem Contamination Monitoring



Chemical Approach:

Chemicals concentrations of metals, aromatic hydrocarbons, pesticides, pharmaceuticals by analytical tools (HPLC, GC, MS...)

Biological Approach:

- Molecular, Biochemical and Physiological dysfunctions
- Tissues pathology; Morphology alteration
- Ecological indexes

Assessment of chemicals biodisponibility and its negative effects on organisms health

Biomarkers

Biochemical

MTs, ATPase, GST,
AChE, GPx, P450, etc.

Immunological

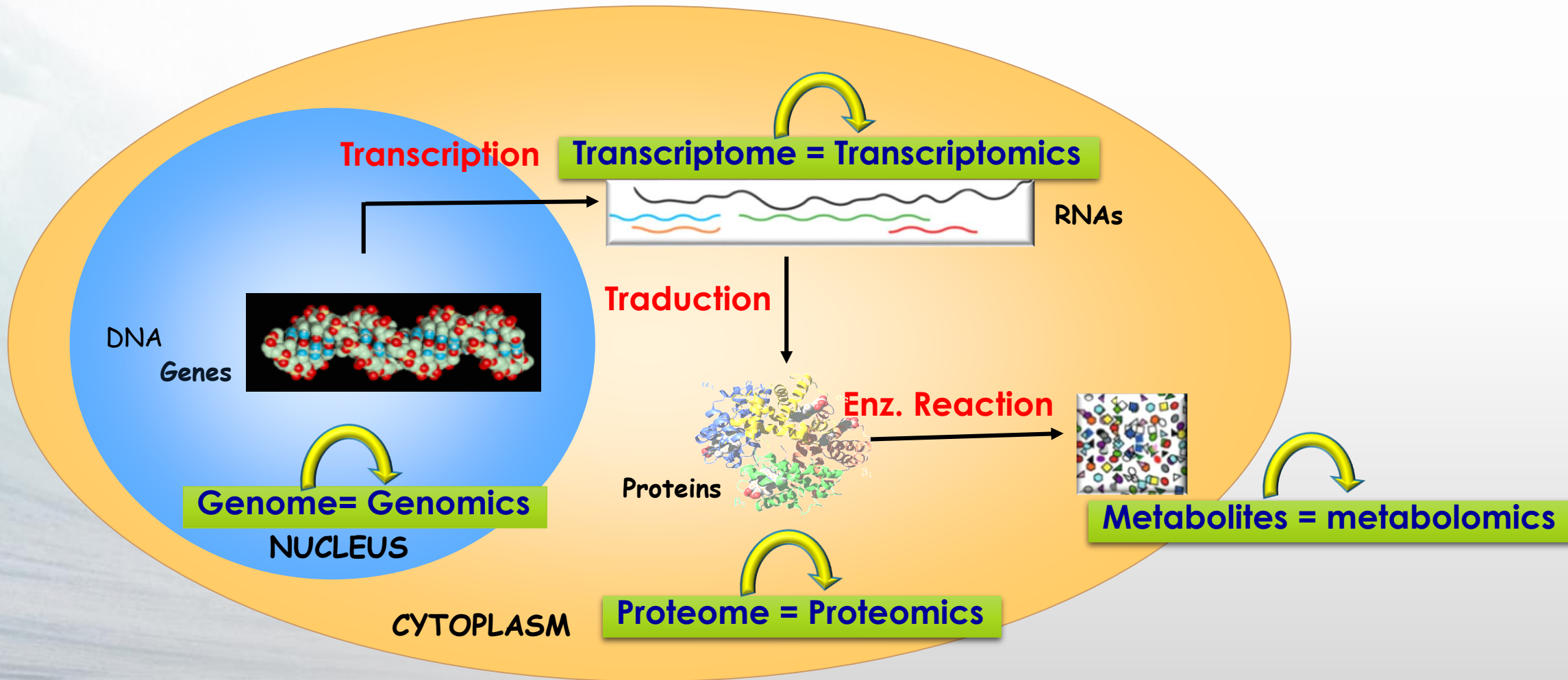
Lysozyme activity
Complement activity
Respiratory burst activity

Reproduction

Sex steroid hormones
Gonadosomatic indices

Environmental Proteomics:

Study of the proteome of organism under environmental stress



Proteomics strategy

Separation of proteins

Quantification

Digestion of protein into peptides

Mass spectrometry

Protein Identification

Tools of Proteomics

2D-Electrophoresis : IEF and SDS-PAGE)

2D-Gels image analysis with PDQuest Software

Trypsin is a serine protease that cleaves protein chain into peptide of 10-20 amino acids

MALDI-TOF: mass of each peptide
ESI-MS-MS: sequence of each peptide

Compare the **mass** or **sequence** of each peptide to those of known proteins availables in the **databases**



**Assessment of biochemical response of
Mediterranean Crab collected from Monastir Bay
(polluted area) and from Kuriat station as control
area**

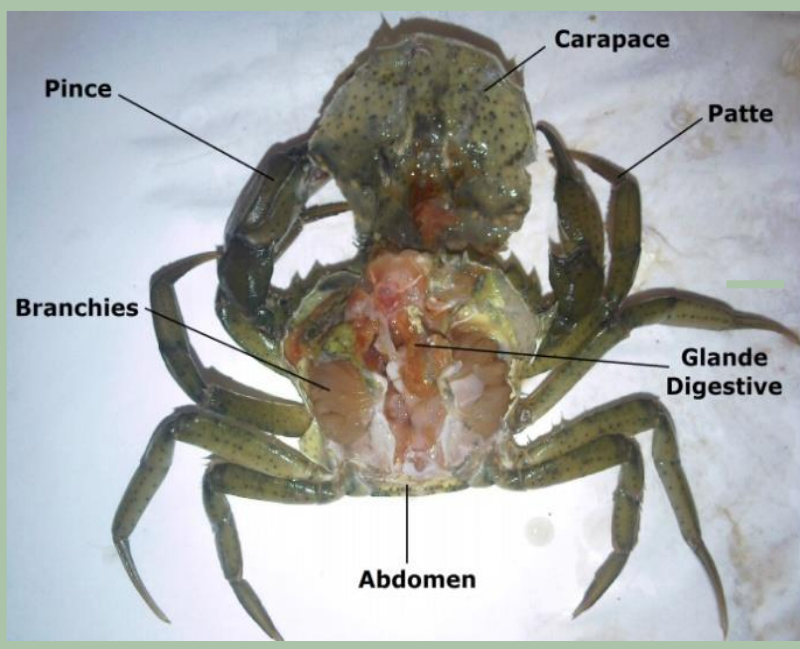
Crabs collection and handling:

Crabs (45–55 mm) were collected at five different selected sites from the Monastir Bay and Kuriat Island as a reference site.

Catalase activity
(CAT)

Malondialdehyde level
(MDA)

glutathion-S-transferase
(GST) activity



Acetylcholinesterase
activity (AChE)

Metallothionein level
(MTs)

Lactate deshydrogenase
activity (LDH)



Libya

200 km

charge

nt

Re

mta

Téboulba

(5

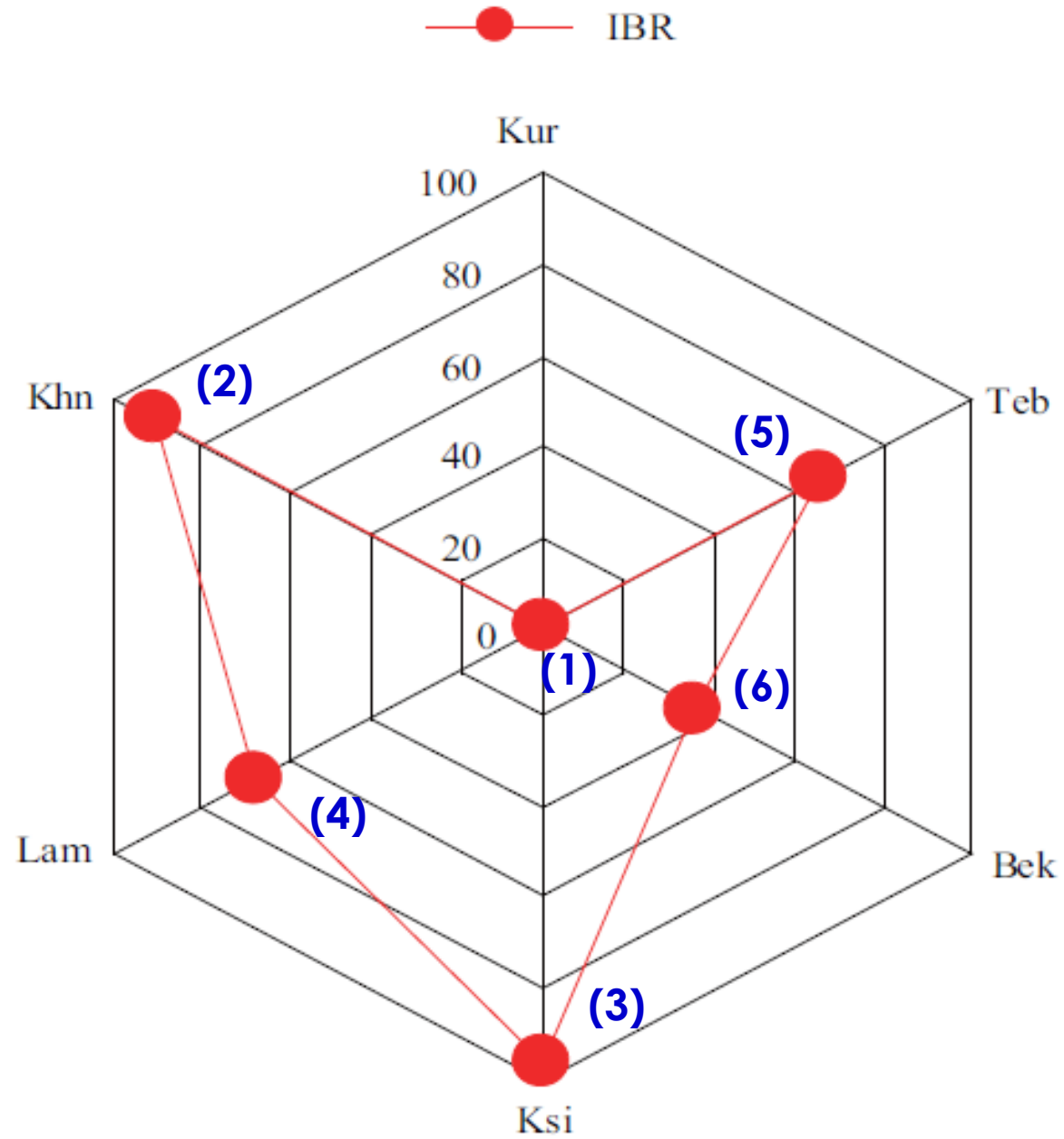
Kuriat



$\mu\text{mol}/\text{min}/\text{mg}$ protein

μg MT/ mg protein

$\text{nmol}/\text{min}/\text{mg}$ protein



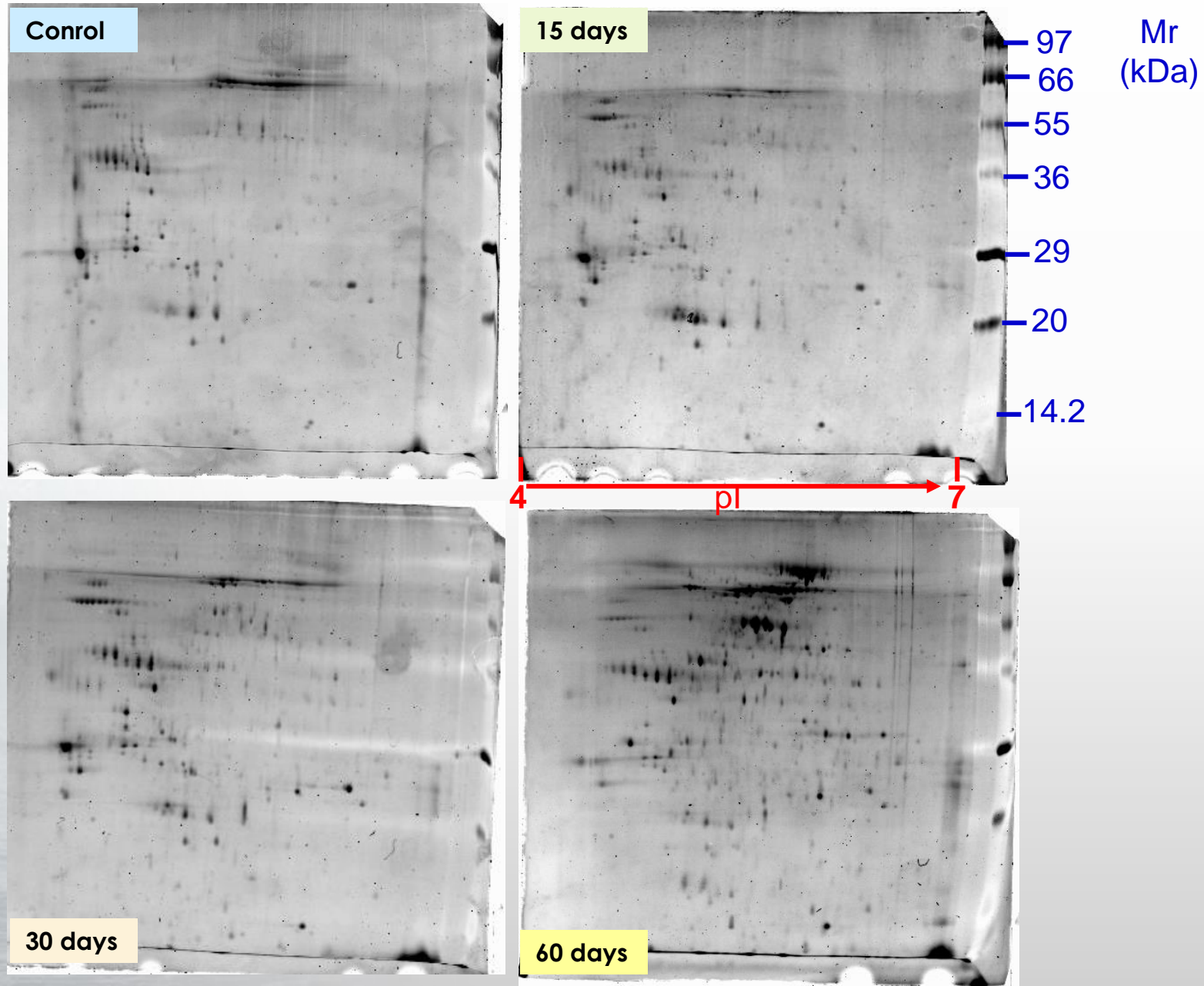


**Assessment of Proteomic response in Mediterranean crab
after their transplantation into the fishing Harbour**

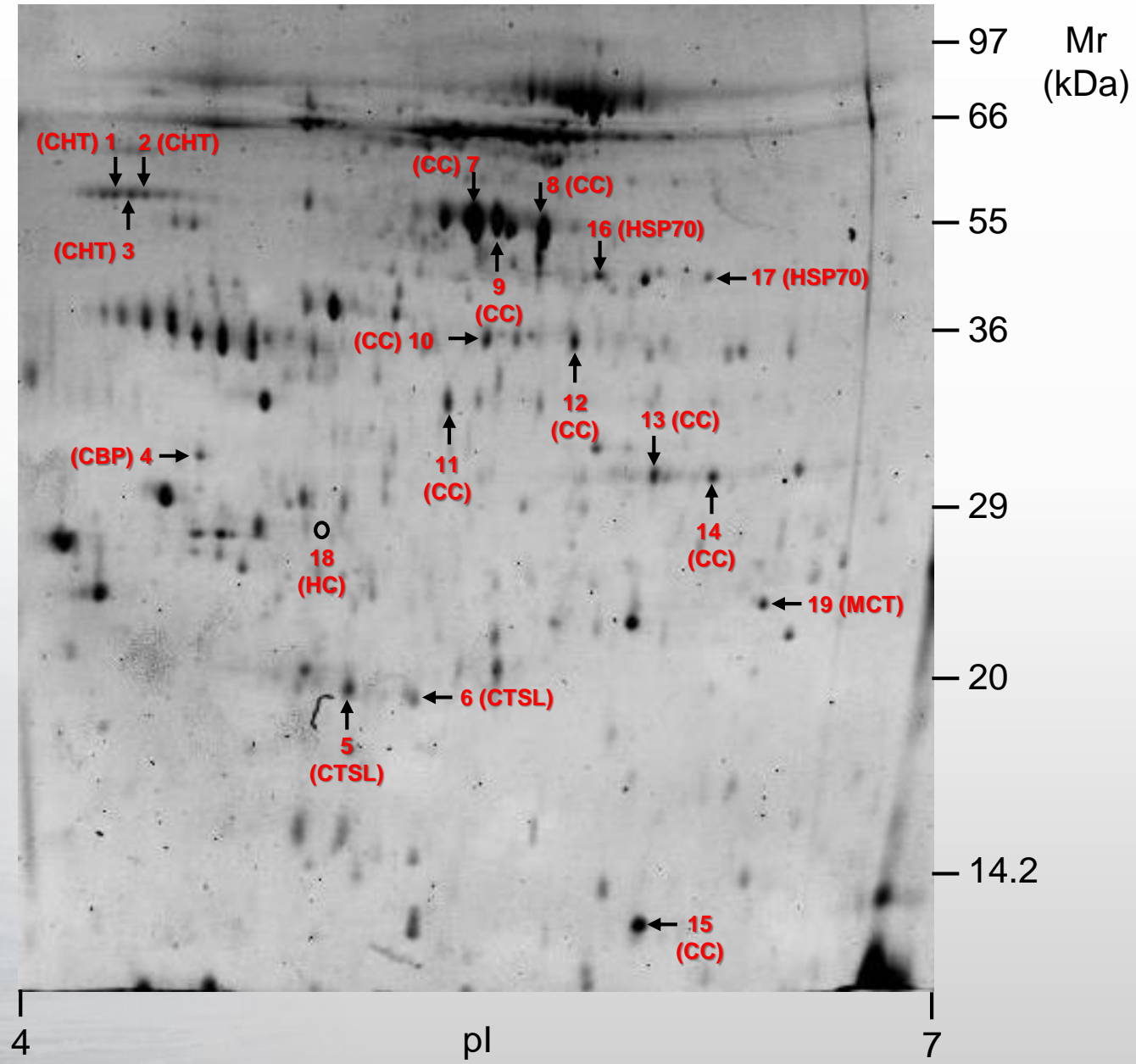
- ✓ **Crabs from uncontaminated area, were placed in a polyethylene netting cage and transferred into the fishing harbour.**
- ✓ **The cage was placed at the down of harbour, and the crabs were regularly fed with sardines.**
- ✓ **After 0, 15, 30 and 60 days, crabs were collected and the hepatopancreas was carefully removed for the proteomic analysis.**

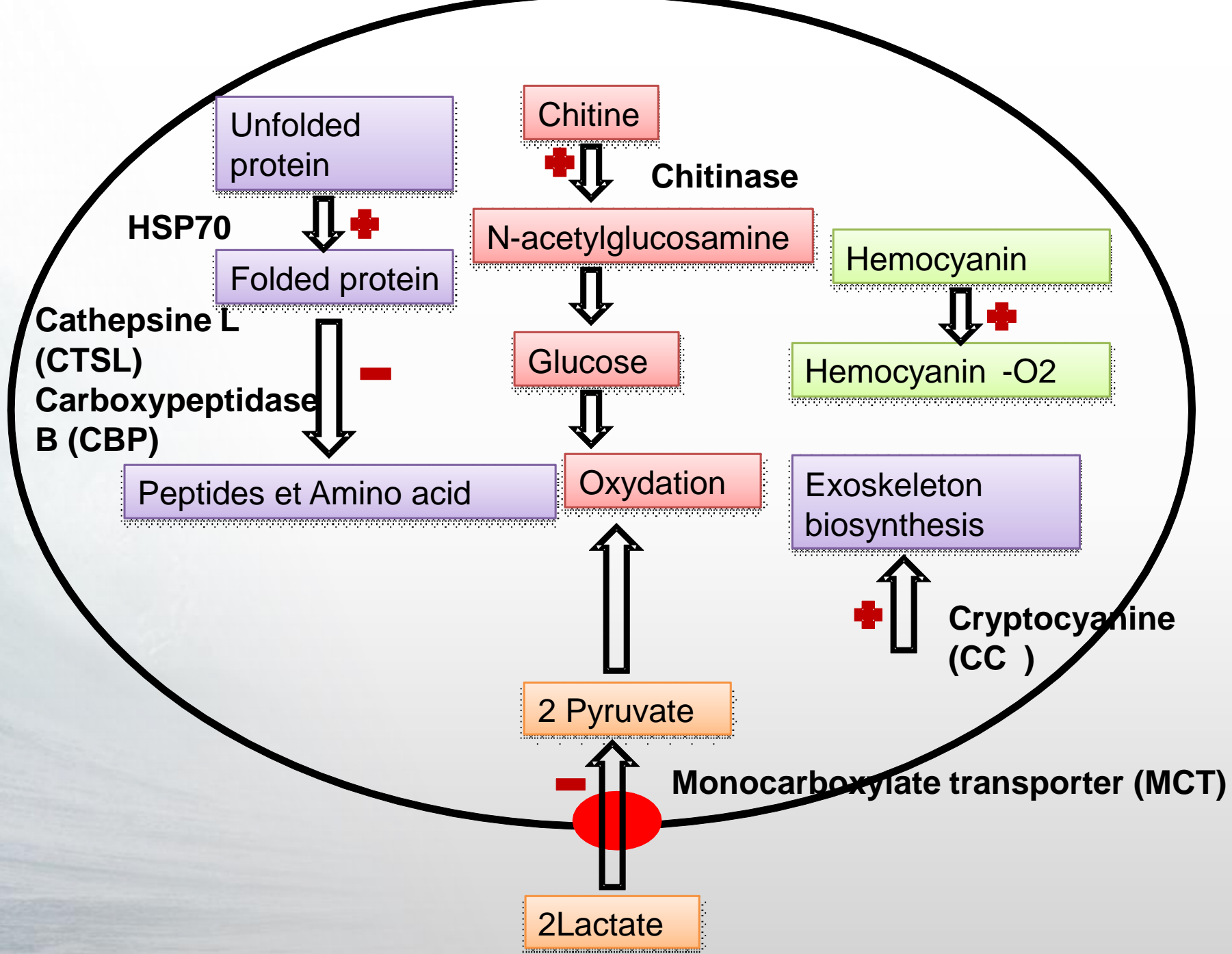


Representative 2-DE gel images of proteins expression of crabs transferred into the Tunisian fishing Harbour for 0, 15, 30 and 60 days.

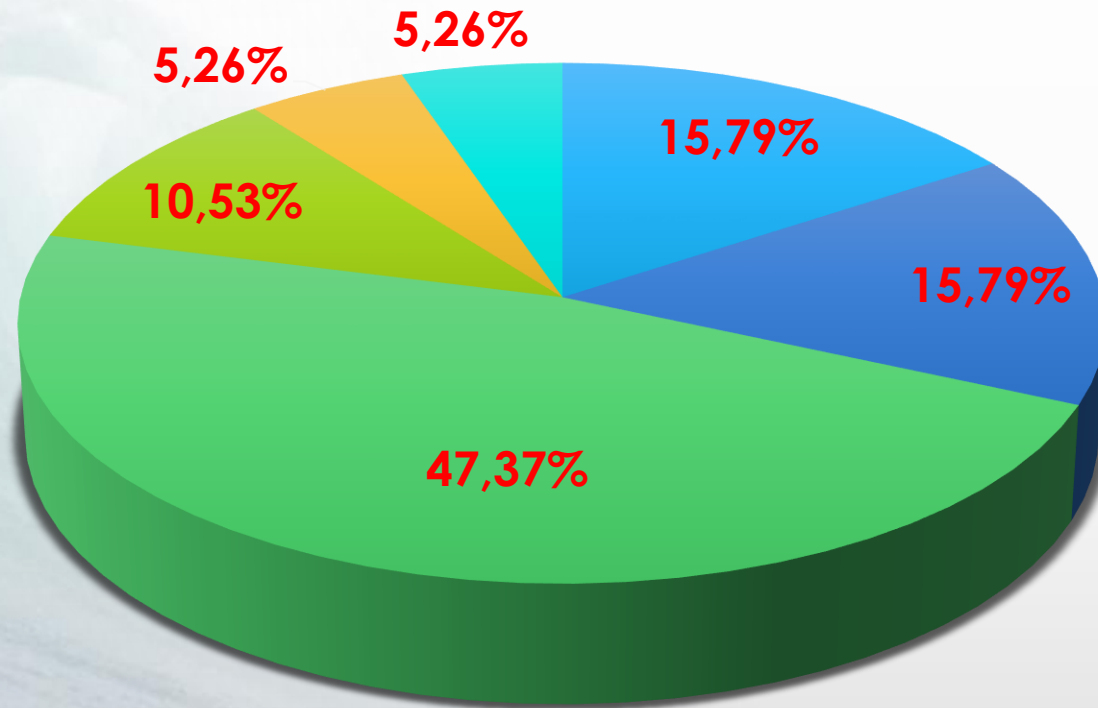


Representative 2-DE gel image showing the 19 differentially expressed spots that were identified by LC-MS/MS.





Identified proteins and their distribution into biological process categories.



■ **Chitin catabolic process**

■ **Proteolysis**

■ **Exoskeleton biosynthesis**

■ **Protein folding, stress response**

■ **O2-Transport**

■ **Transmembrane transport**



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