Supporting Information

Manganese nanoparticle: impact on non-nodulated plant as a potent enhancer in nitrogen metabolism and toxicity study both in vivo and in vitro

Saheli Pradhan [a], Prasun Patra [b], Shouvik Mitra [a], Kushal Kumar Dey[c], Sneha Jain[a], Samapd Sarkar[a], Shuvrodeb Roy[a], Pratip Palit[d], Arunava Goswami [a]*

[a]Biological Sciences Division, Indian Statistical Institute, 203 B.T. Road, Kolkata 700108, India.

[b] Centre for Research in Nanoscience and Nanotechnology, University of Calcutta, Kolkata,700098,India.

[c]Department of Statistics, University of Chicago

[d]Plant Physiology Section, Central Research Institute for Jute and Allied Fibres, Indian Council of Agricultural Research, Barrackpore, Kolkata 700 120, India

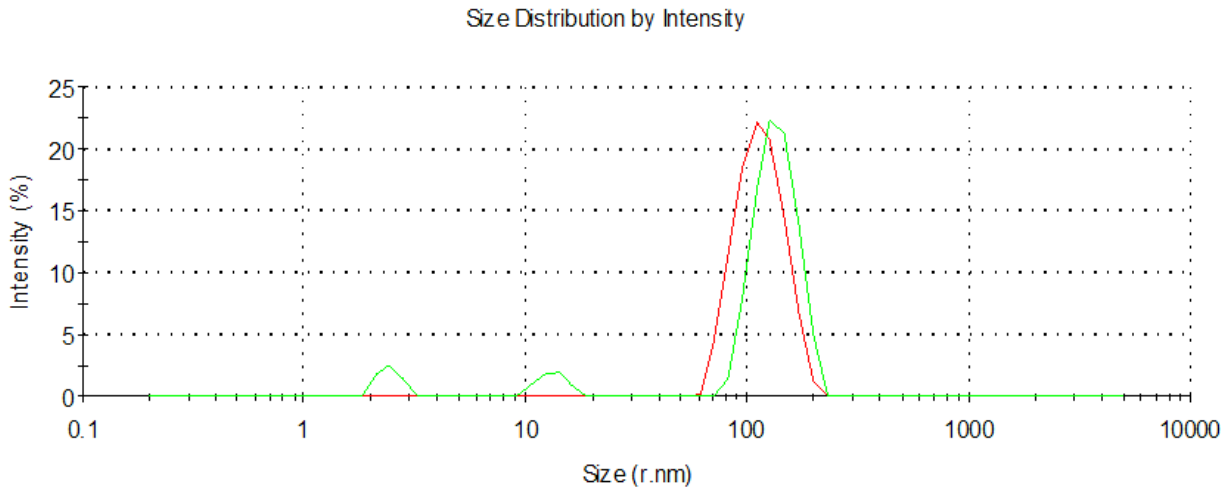


Fig. S1. Hydrodynamic radius measurements of MnNPs dispersion after 12hr (red line) and 24hr (green line). No significant changes were noted between the two exhibiting a stable dispersion.

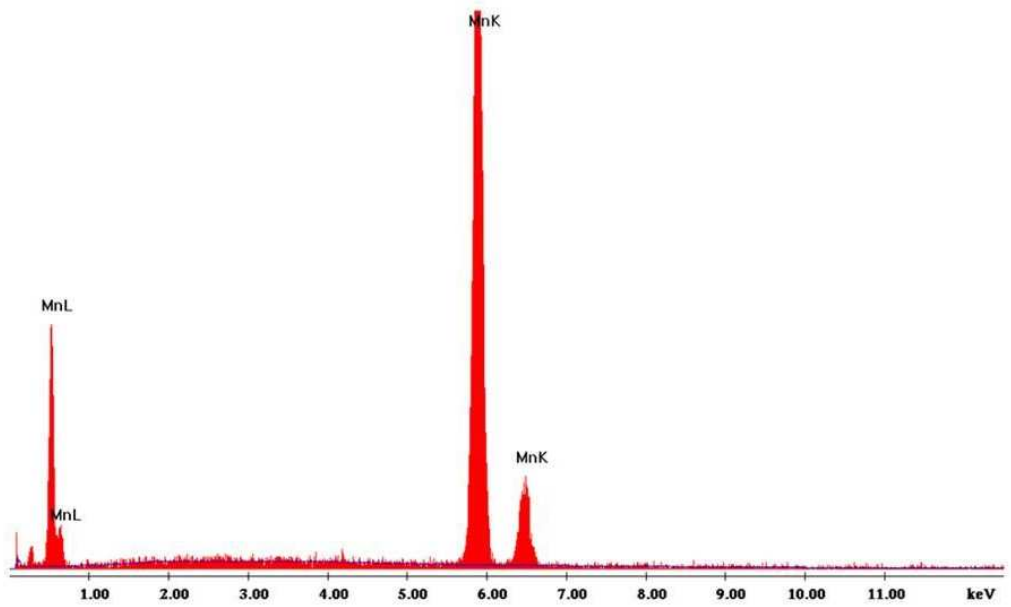


Fig. S2. EDX spectrum of MnNPs, which showed Mn was present as the main chemical components.

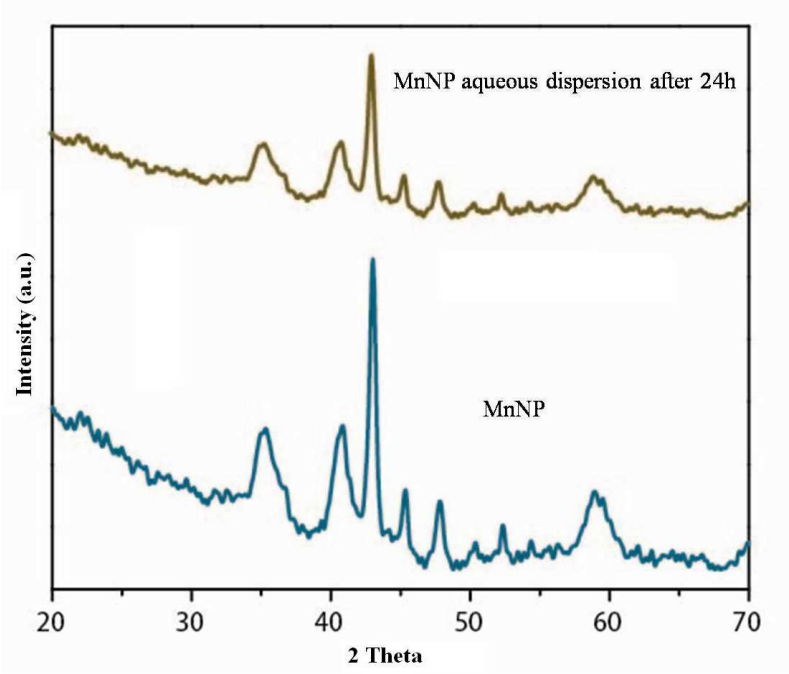


Fig. S3. XRD pattern of MnNPs and MnNPs dispersion after 24hr. No significant changes were noted in the pattern which claimed its stability.

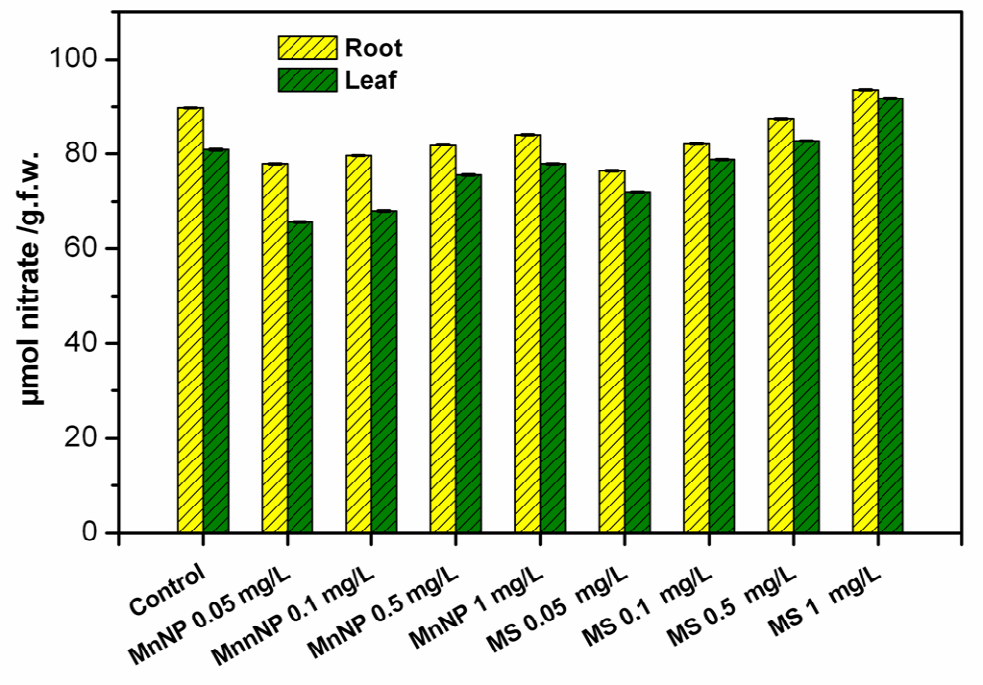


Fig. S4. Effect of MnNP and MS on NO_3^- concentration of 15days treated mung bean plants.

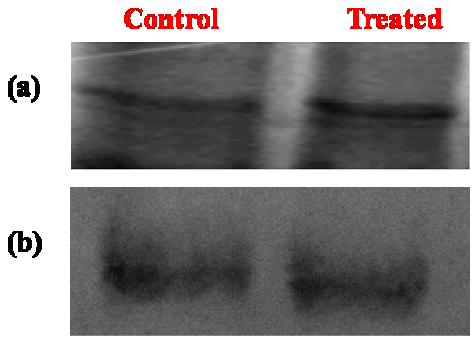


Fig S5. (a) SDS-PAGE analysis of isolated NR protein from control and treated leaf tissue, (b) Western blot images of isolated NR protein of control and treated leaf tissue of mung bean plant.

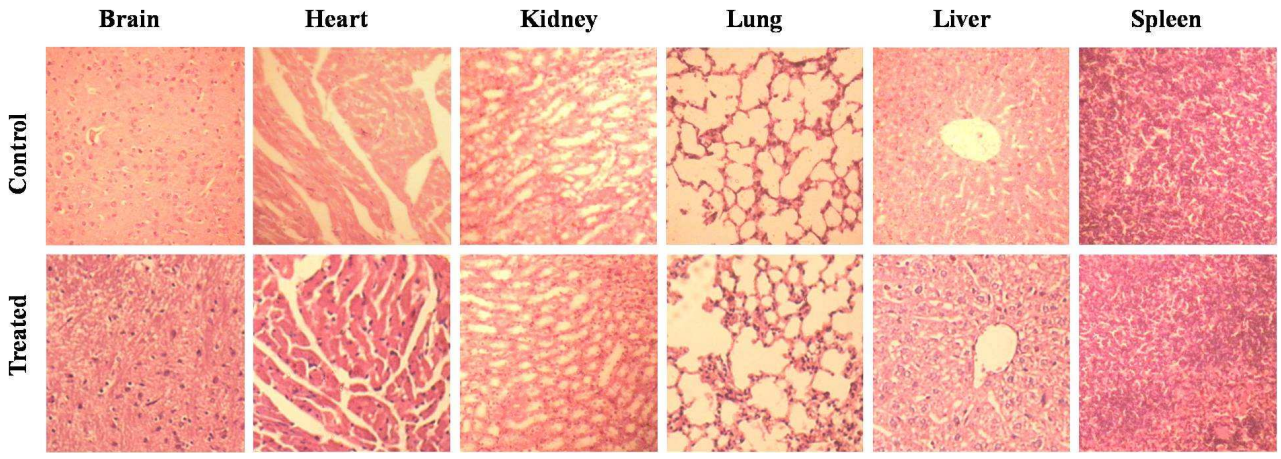


Fig. S6. Histological analysis of MnNP treated major organs of mice.

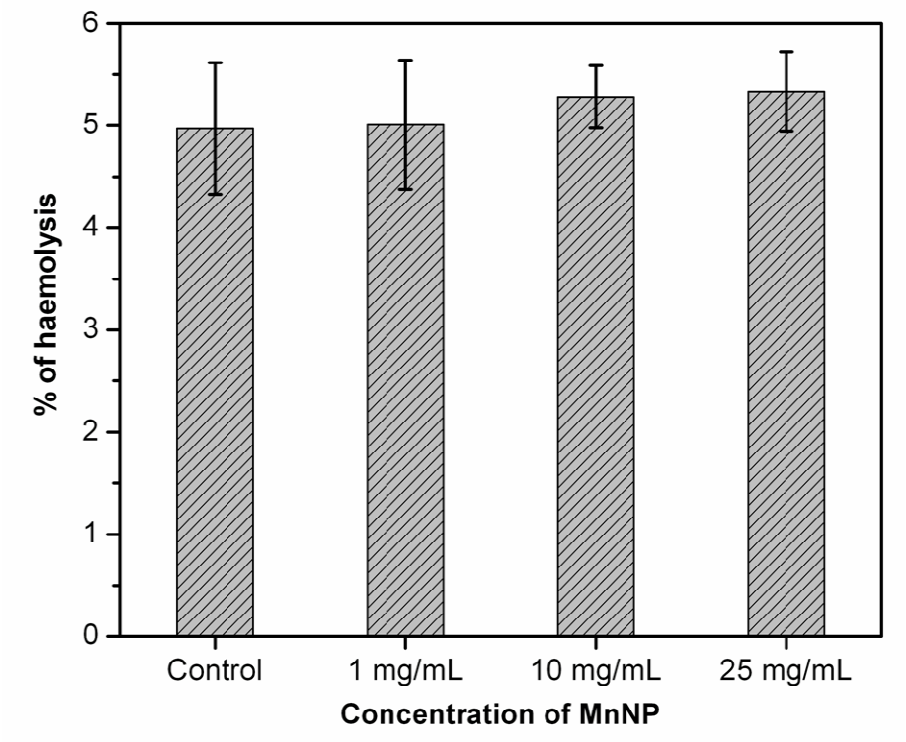


Fig. S7. Effect of MnNP on haemolysis of human blood samples.

SI Table 1: Body weight of MnNP treated and untreated (control) mice in intraveinous toxicity assay.

Animal	Control	Mn-1mg/L	Mn-10mg/L	Mn-25mg/L
Mice body weight (gm)	20.933 ±1.629	19.967 ±1.604	21.867 ±1.387	21.167 ±1.739

Parameters		Control	MnNP		
			1mg/L	10mg/L	25mg/L
Hemoglobin (g/dl)		15.57± 0.8145	15.1667± 0.1528	15.067± 0.2517	15.133± 0.01528
Total Count	**RBC (million/ mm^3)**	5.03±0.5686	4.767± 0.2517	4.9±0.2646	5.0333±.1528
	WBC(million/ mm^3	6150±217.9449	6133.333±208.17	5333.333±152.75	5933.33±404.15
Differential Count (%)	**Neutrophils**	39±3.6056	39.667±4.163	40±2	39.33±6.6583
	Lymphocytes	25±2	33.67±4.16	35.33±2.082	33±2
	Monocytes	2±0	2±0	2±0	2±0
	Eosinophils	2±0	2±0	2±0	2.667±0.5774
	Basophils	0±0	0±0	0±0	0±0
Plt (lakh/ mm^3)		1.62±0.02	1.6±0.01	1.79±0.05	1.63±0.022
LDH (IU/L)		224.333±15.63	223±11.53	231.67±10.41	215.67±6.028
Creatinine (mg/dl)		0.85±0.025	0.88±0.027	0.91±0.04	0.93±0.04
ALP (U/L)		71.33±3.21	71±2	72±3	70.33±1.53
Total Protein (gm/dl)		6.83±0.15	7.033±0.15	6.93±0.25	6.8±0.26
Cholesterol (mg/dl)		153.33±11.06	151.07±12.22	155±7	154±6.557
Triglyceride (mg/dl)		92.67±2.52	92±6.08	90.33±5.51	103.33±10.41
Uric Acid (mg/dl)		6.43±0.40	6.27±0.25	6.77±0.15	6.63±0.252
BUN (mg/dl)		11±1	12±2	12.67±1.53	12.33±1.528
SGOT (Unit/L)		33.33±3.51	34.33±3.2	35.67±2.08	35.67±3.055
SGPT (Unit/L)		30.33±3.01	32.67±1.53	31±2	35.33±2.517
Phosphorous (mg/dl)		3.53±0.25	3.1±0.26	3.26±0.31	3.37±0.15

SI Table 2 Comparison of blood biochemical parameters between control and MnNP treated mice.

For the final dataset that was analysed, we observed that only two of the characteristics were significant as per the ANOVA test- they are Platelets and WBC. We performed Tukey's post-hoc test on these two cells and the following table presents the analysis

Platelets

Control	Mn 1 ppm	Mn 10 ppm	Mn 25 ppm
A	A	B	C

WBC cells

Control	Mn 1 ppm	Mn 10 ppm	Mn 25 ppm
A	A	B	C

The above result is quite intuitive from the box plot of the Platelets and the WBC cells attached.

Experiments	**F val**	**P val**
Haemoglobin	0.79	0.53
RBC	1.47	0.29
WBC	6.32	0.02
Neutrophil	0.03	0.99
Lymphocyte	8.58	0.01
Platelet	28.59	0.00
LDH	0.99	0.45
Creatinine	2.25	0.16
ALP	0.23	0.88
Total protein	0.74	0.56
Cholesterol	1.12	0.40
Triglyceride	2.31	0.15
Uric acid	1.85	0.22
BUN	0.64	0.61
SGOT	0.43	0.74
SGPT	2.71	0.12

Statistical analysis of blood pathological analysis of MnNP treated mice after intraveinous injection

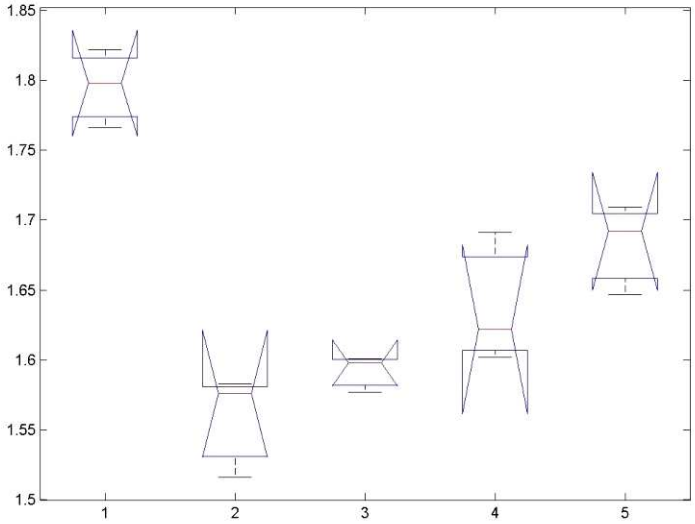
1.

Box plot of the statistical analysis of all biochemical studies with control, MnNP and MS treatments

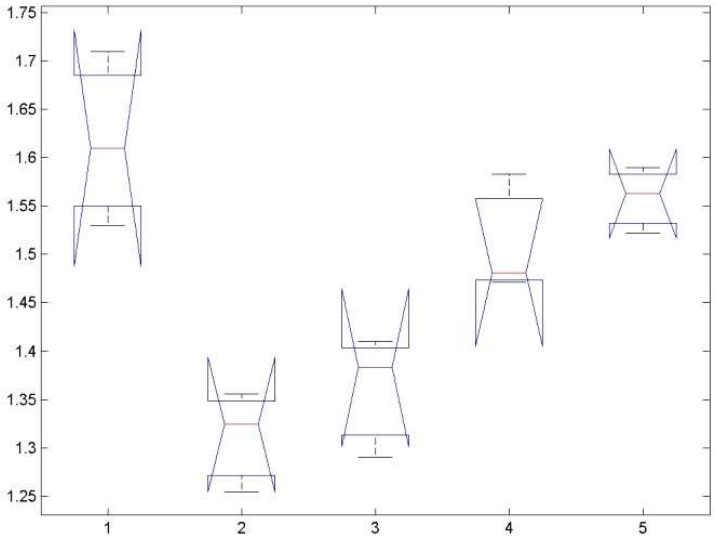
In case cases 1=control, 2=0.05 mg/L, 3=0.1 mg/L, 4=0.5 mg/L, 5=1 mg/L (biochemical tests in plants) and 1=control, 2=1ppm, 3=10ppm and 4=25ppm (biosafety experiments)

1. **Determination of NO_3^-**

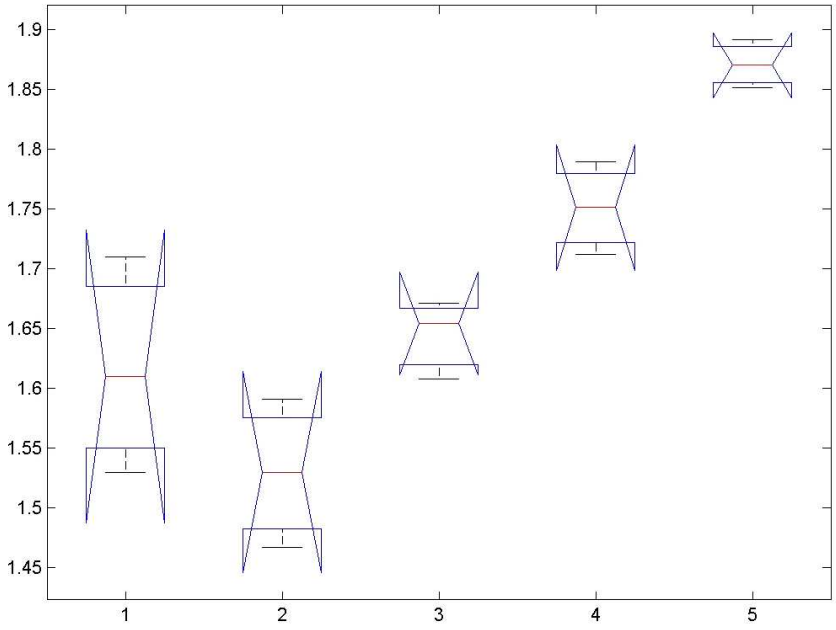
- MnNP leaf



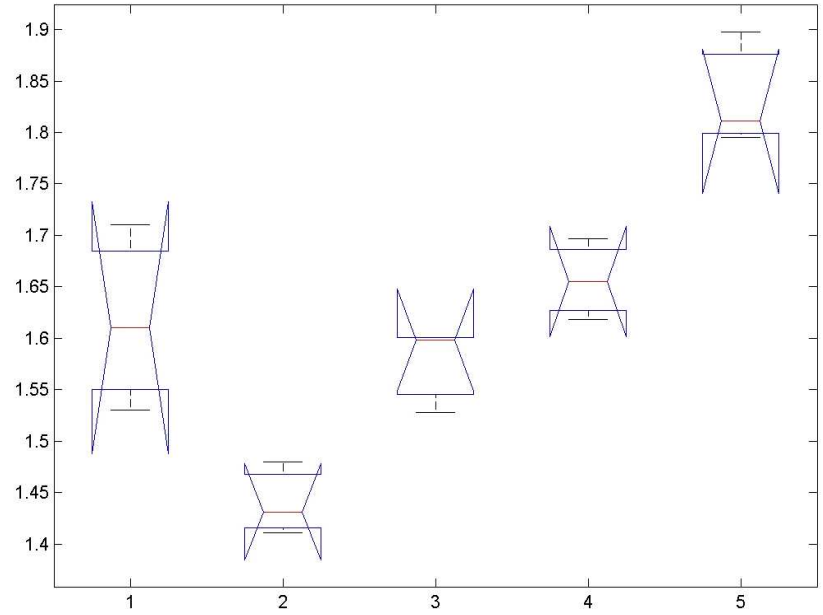
- MnNP root



- MS leaf

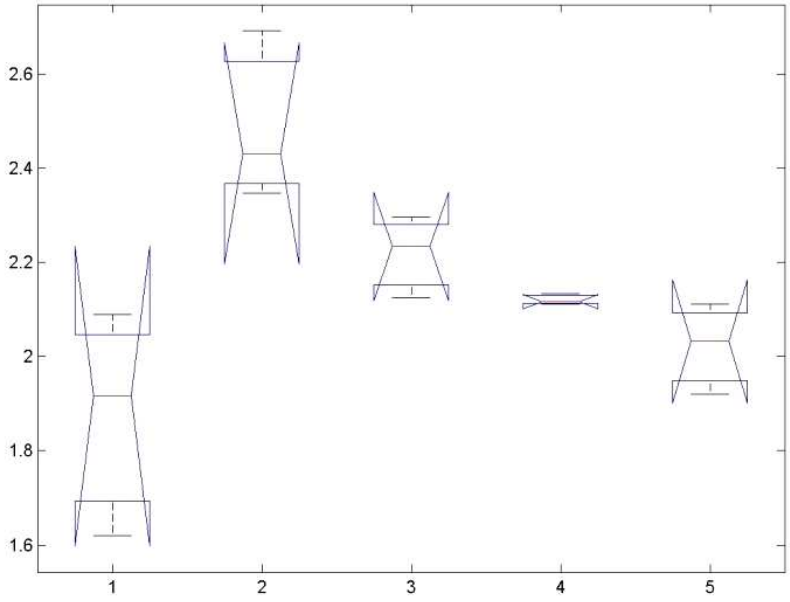


- MS root

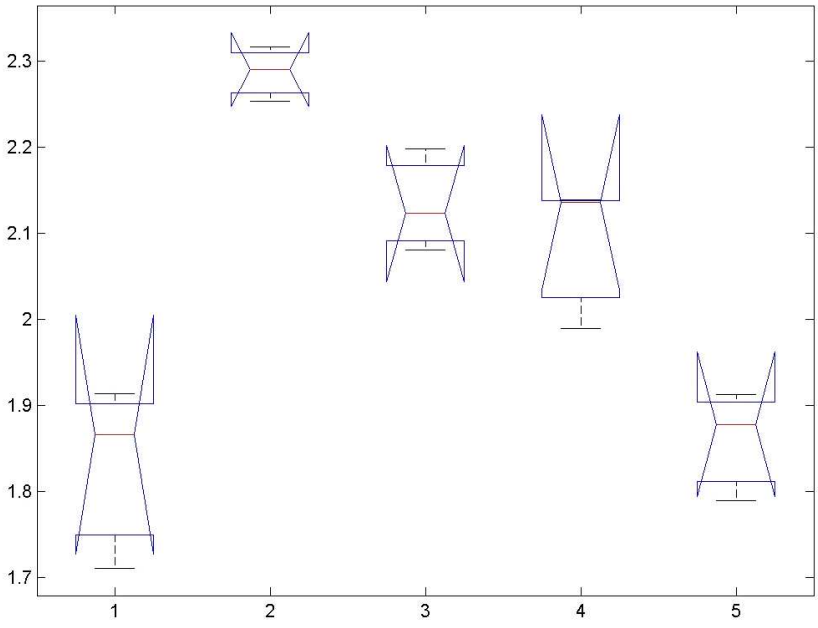


2. Determination of nitrate Reductase (NR) activity

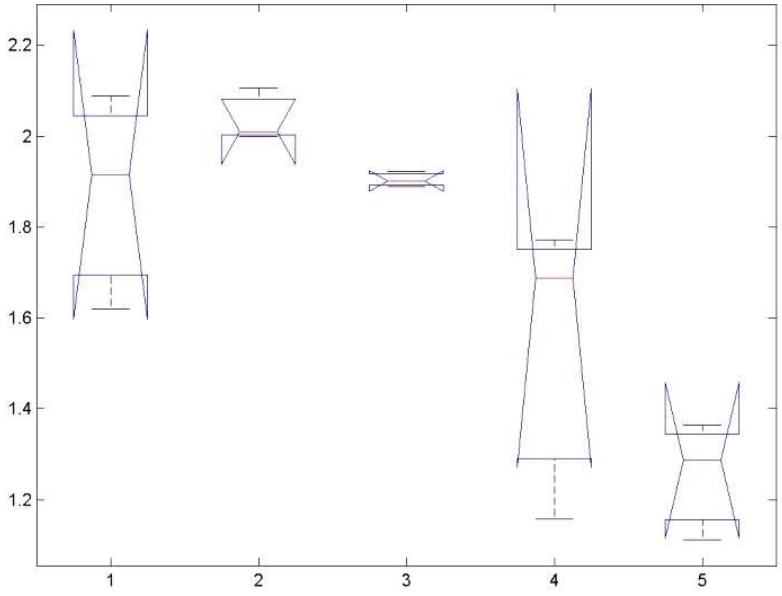
- MnNP leaf



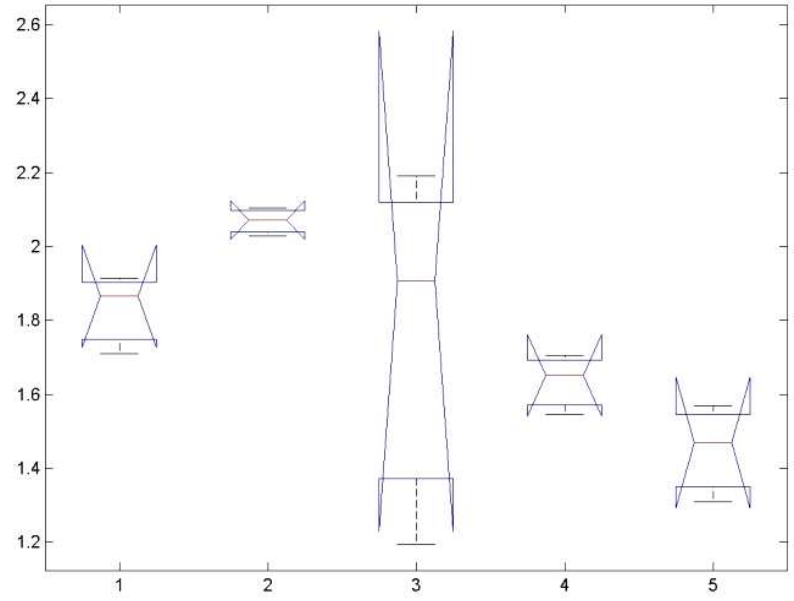
- MnNP root



- MS leaf

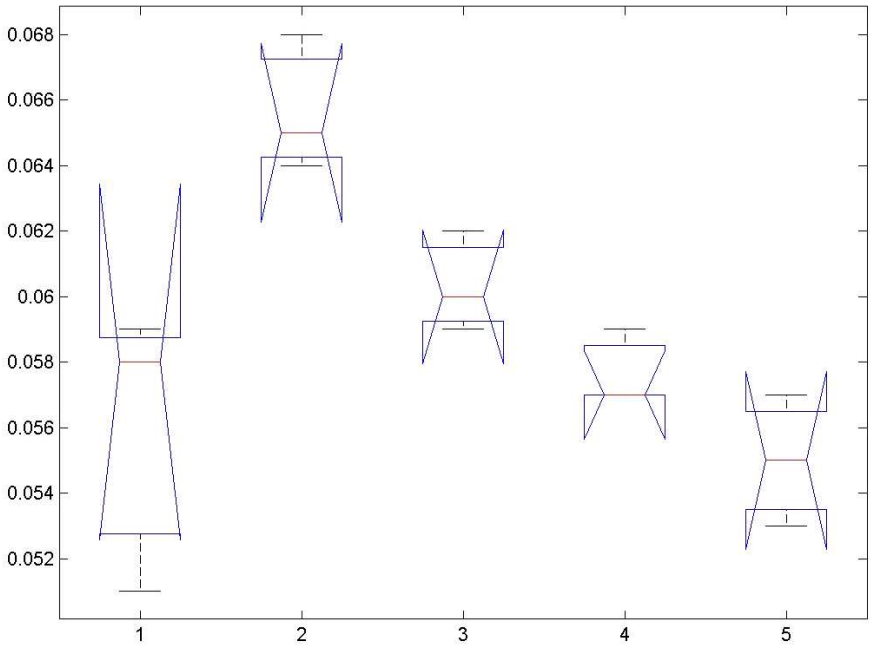


- MS root

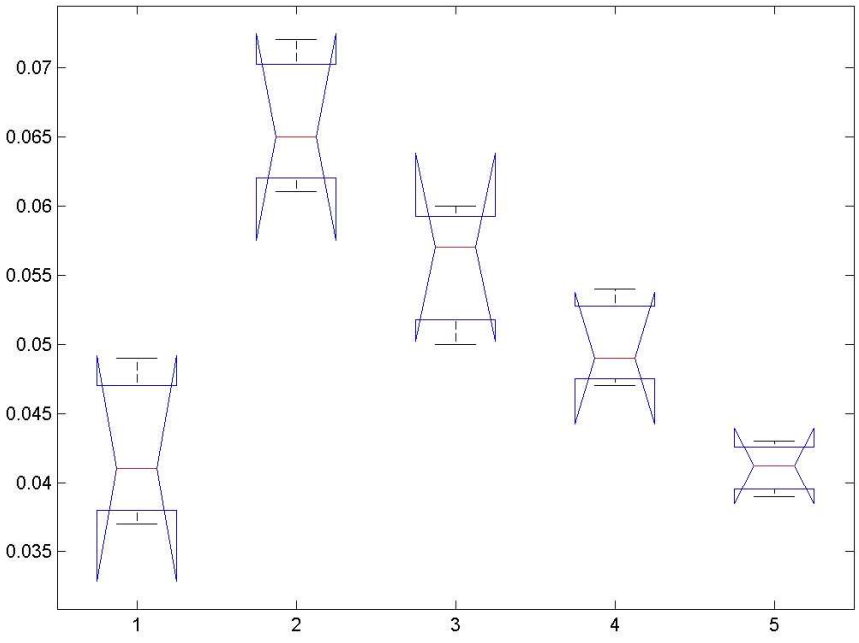


3. Determination of nitrite reduction (NiR) activity

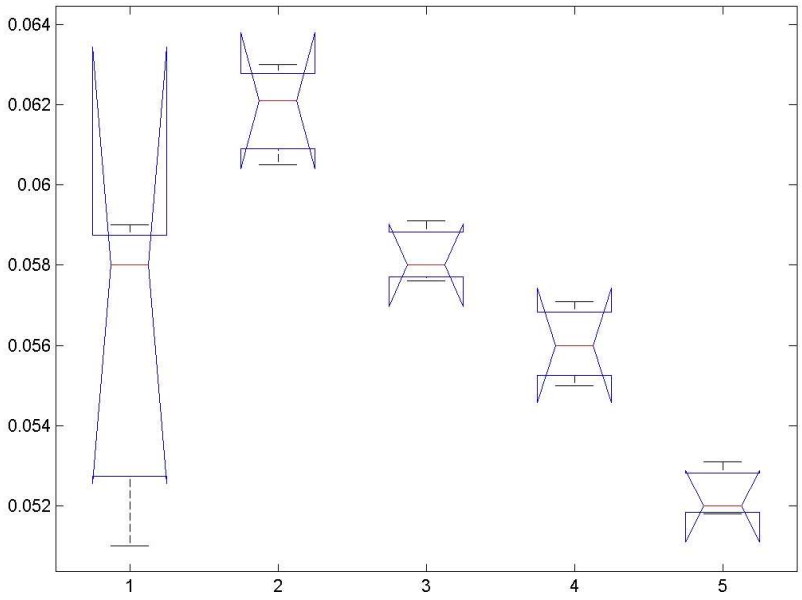
- MnNP leaf



- MnNP root



- MS leaf



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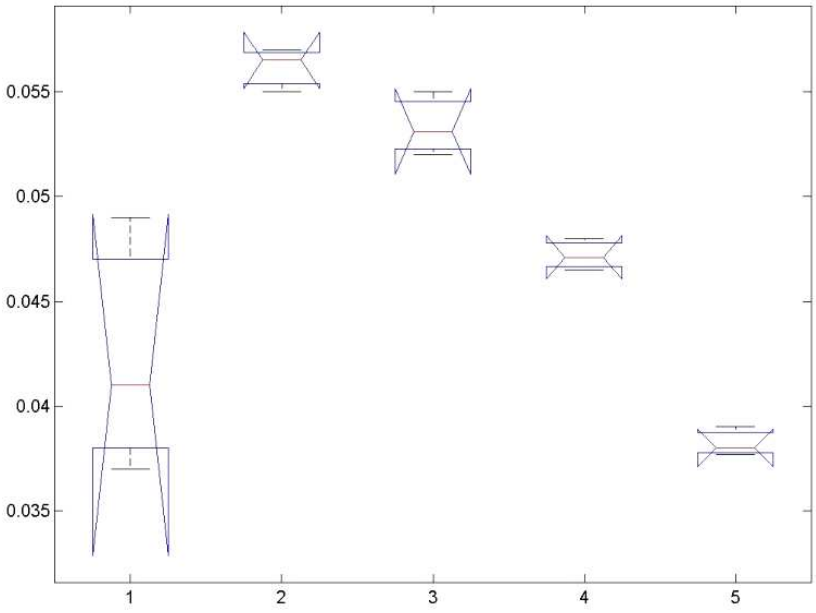
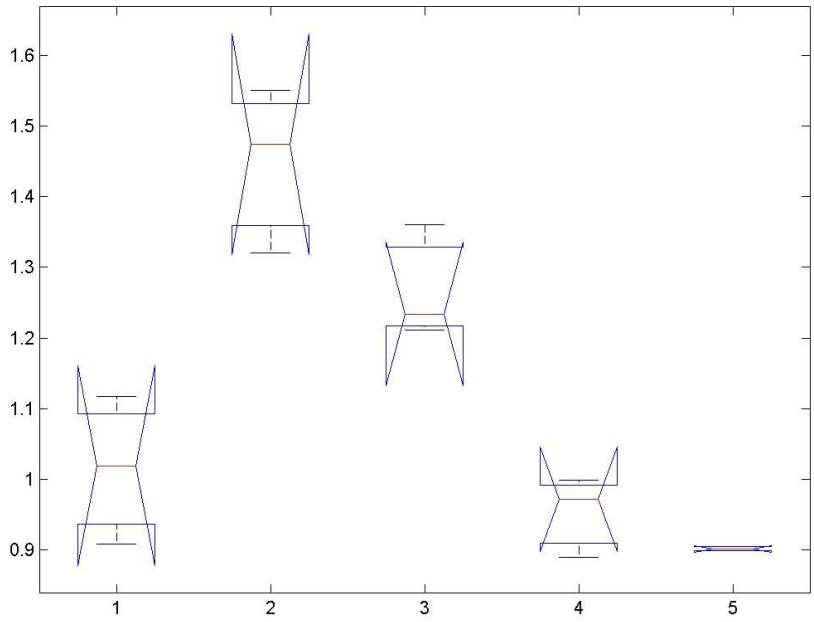
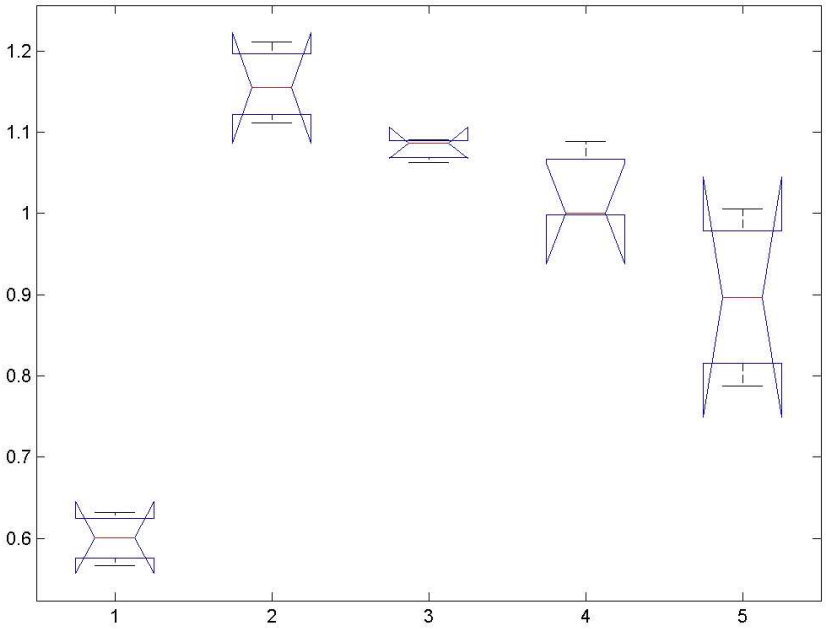


4. Determination of glutamine synthetase (GS) activity

- MnNP leaf



- MnNP root



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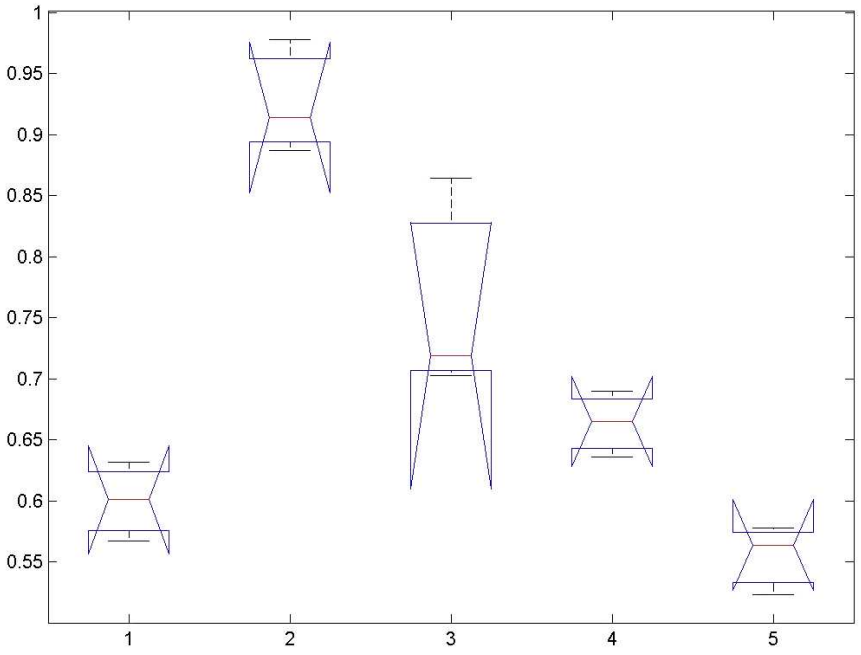
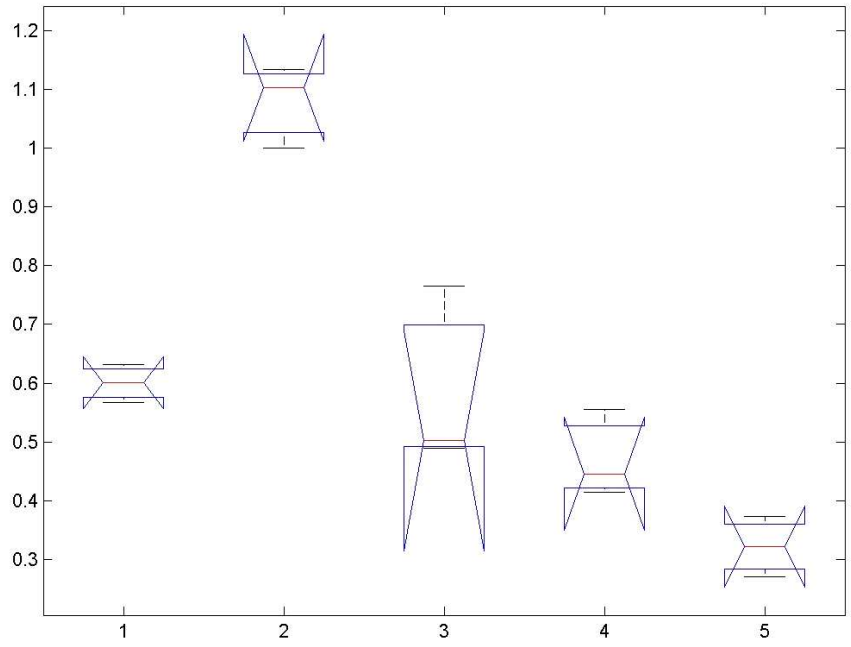
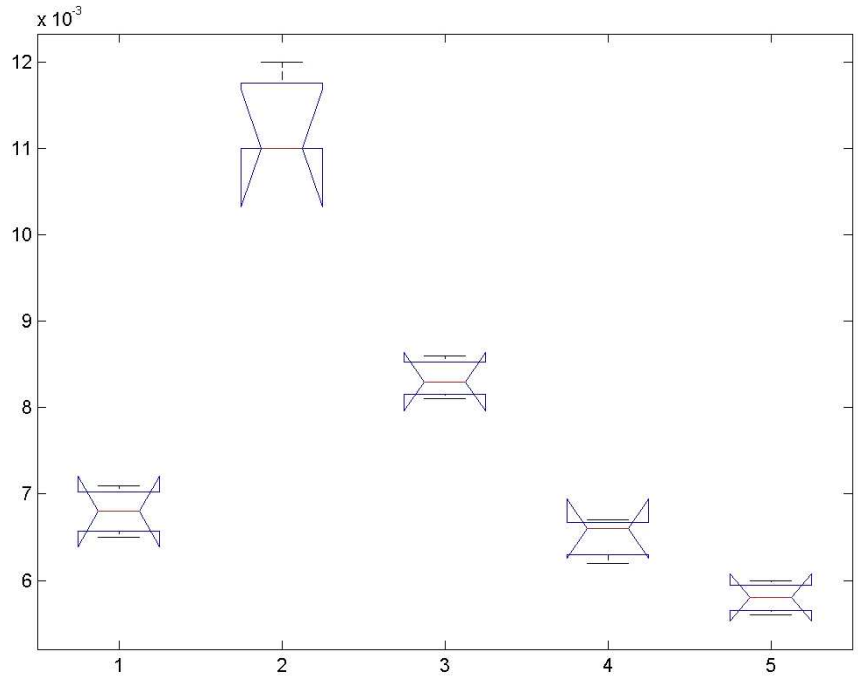


- MS root

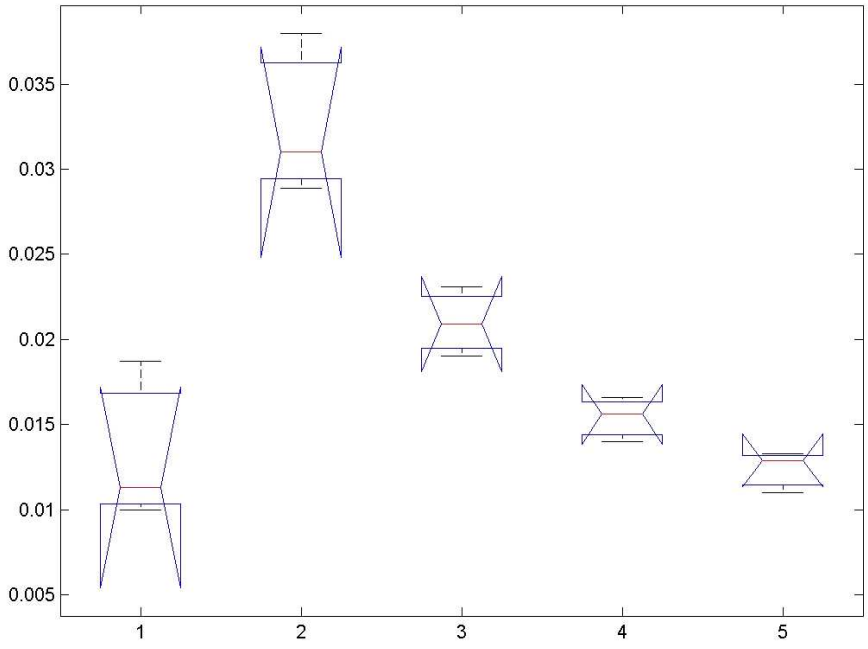


5. Determination of NADH-glutamate synthase (GOGAT) activity

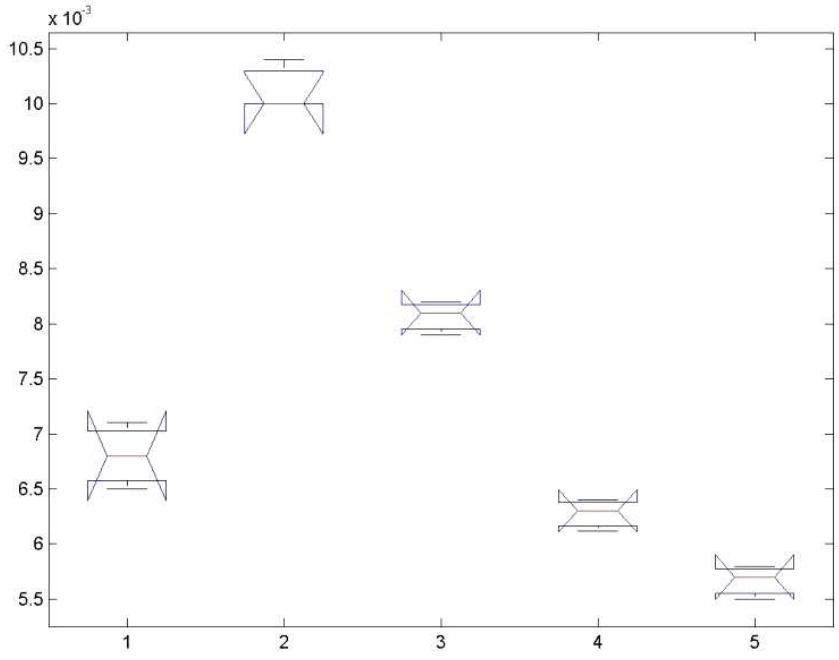
- MnNP leaf



- MnNP root



- MS leaf



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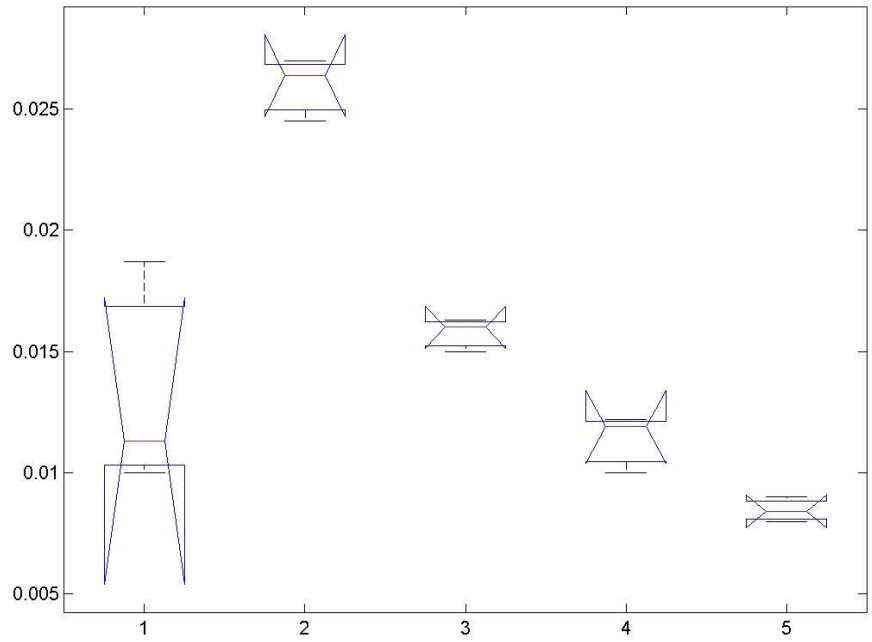
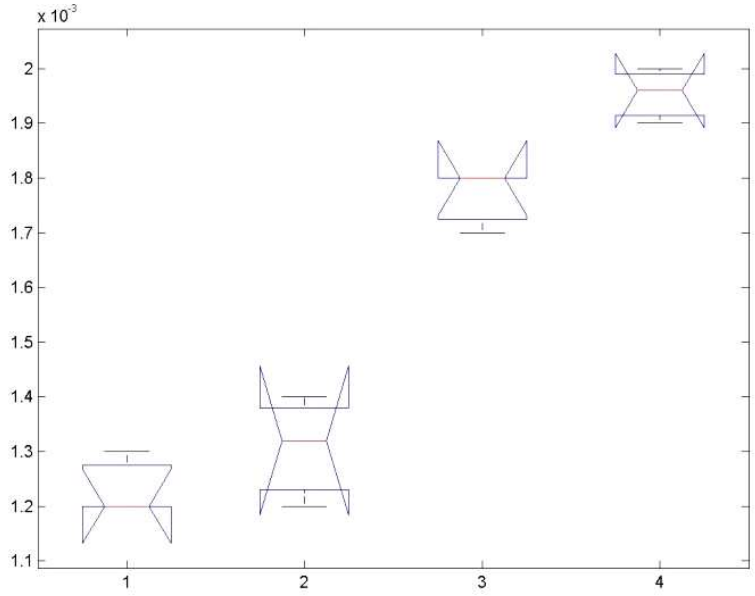
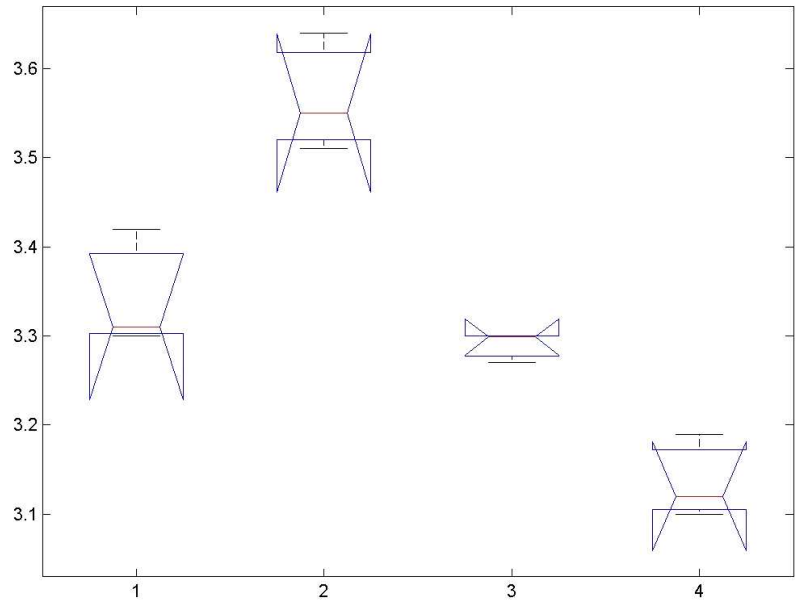


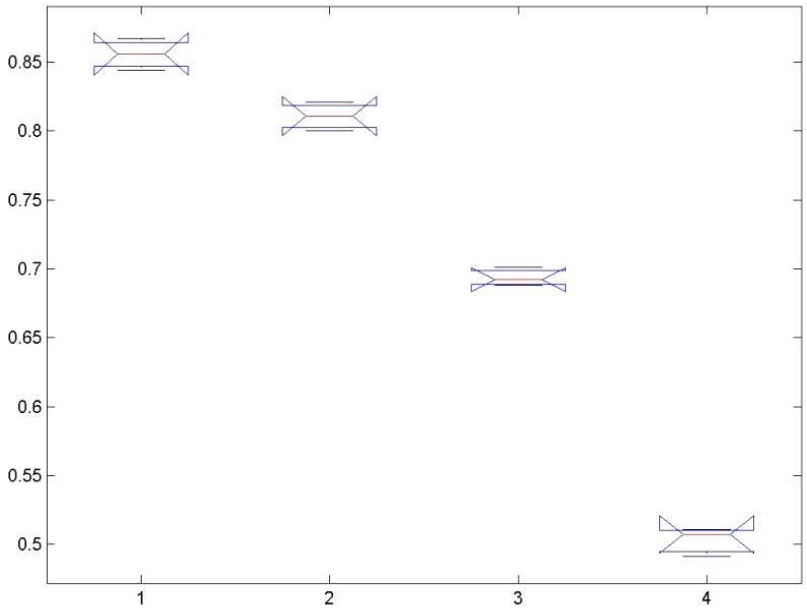
6. Measurement of phosphate utilization of mitochondria



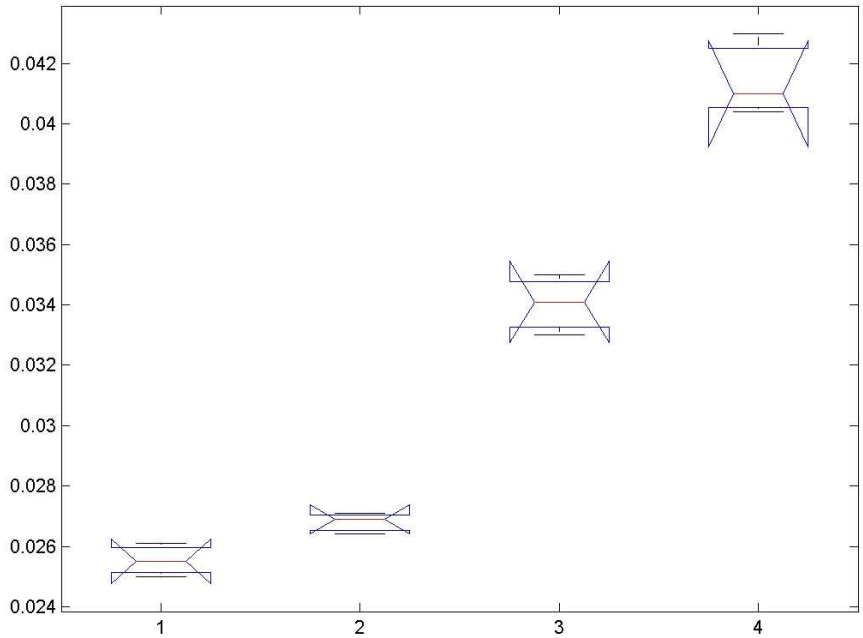
7. Complex I activity



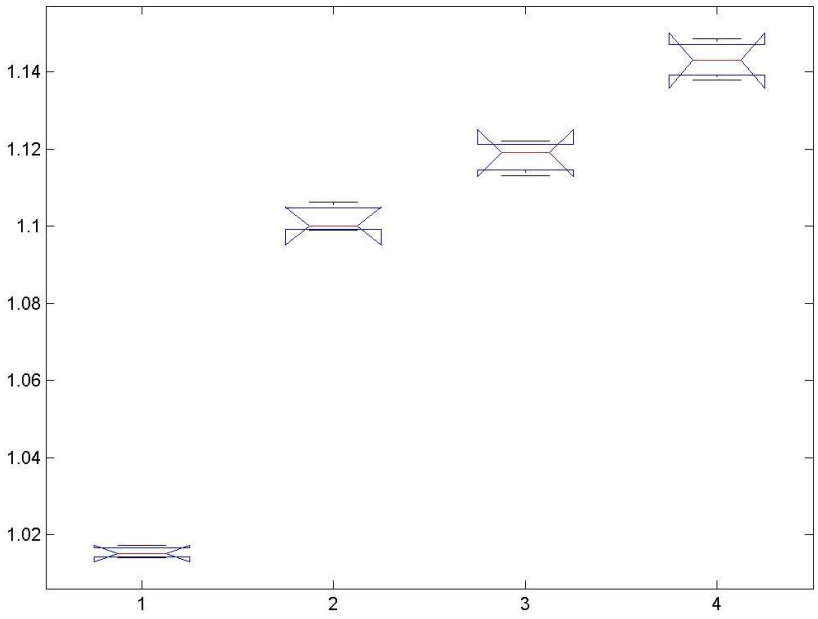
8. Complex II-III activity



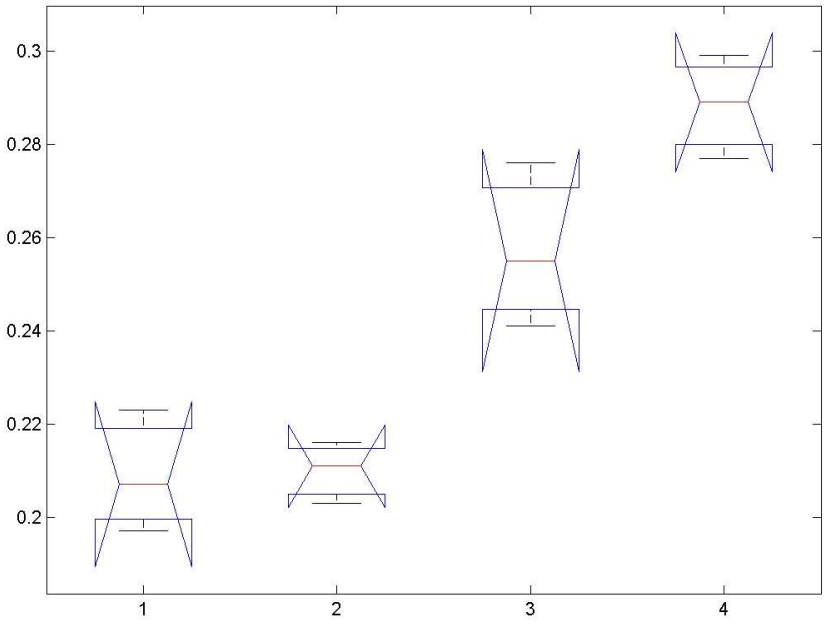
9. Complex IV activity



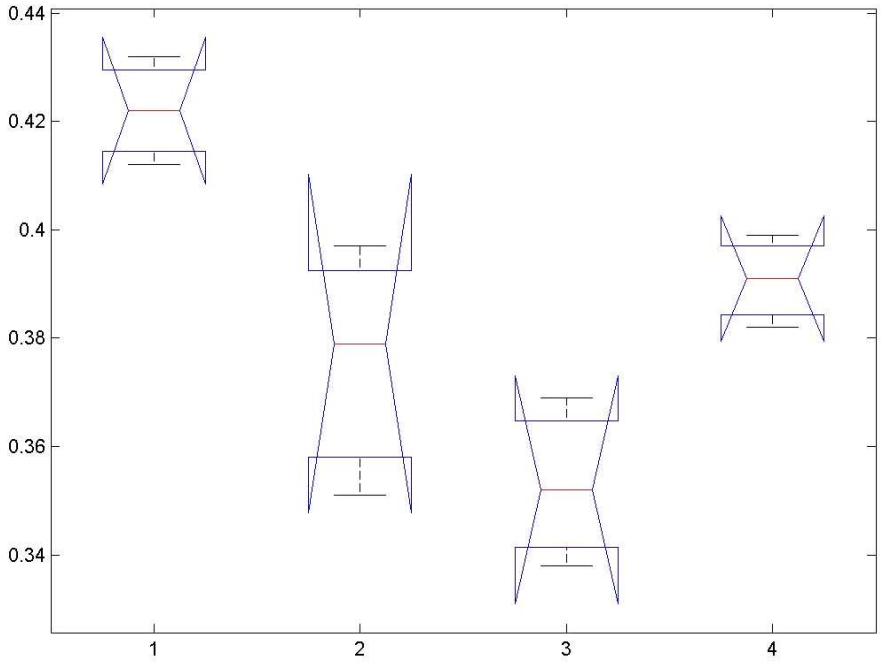
10. Mitochondrial respiratory control ratio (RCR)



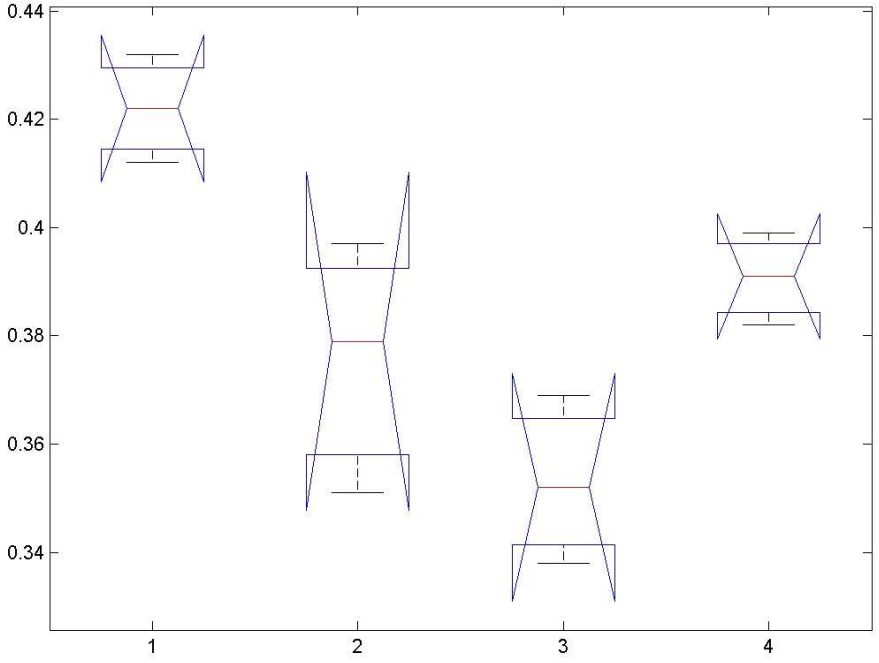
11. Measurement of ROS



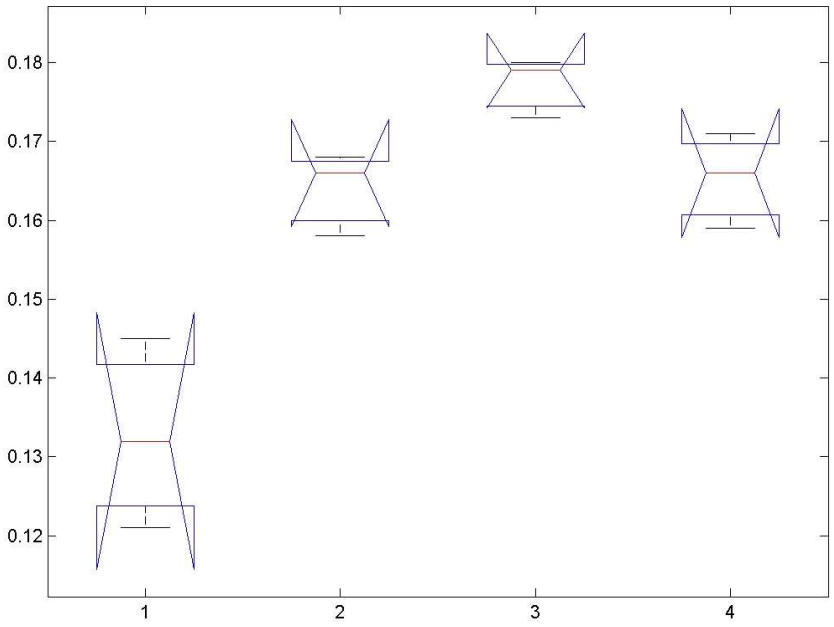
12. WST assay



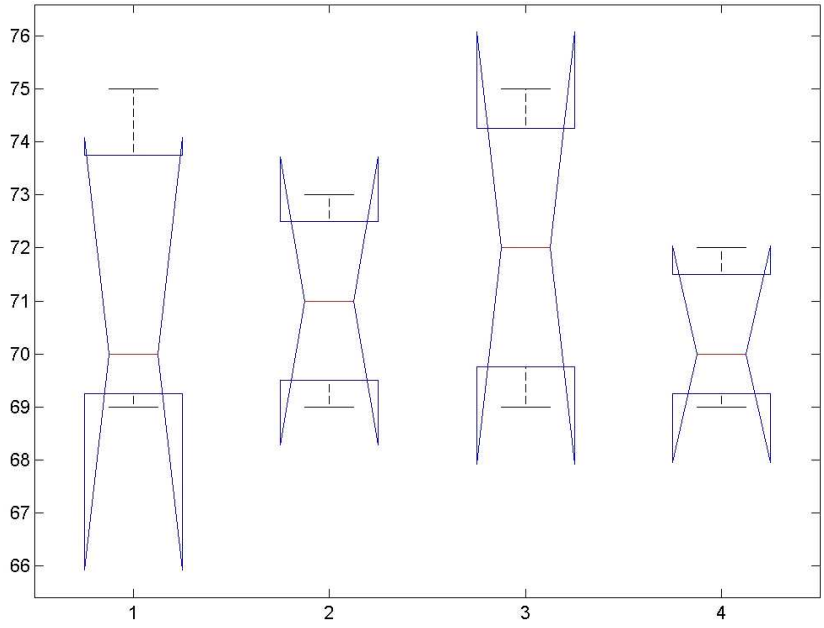
13. NO content



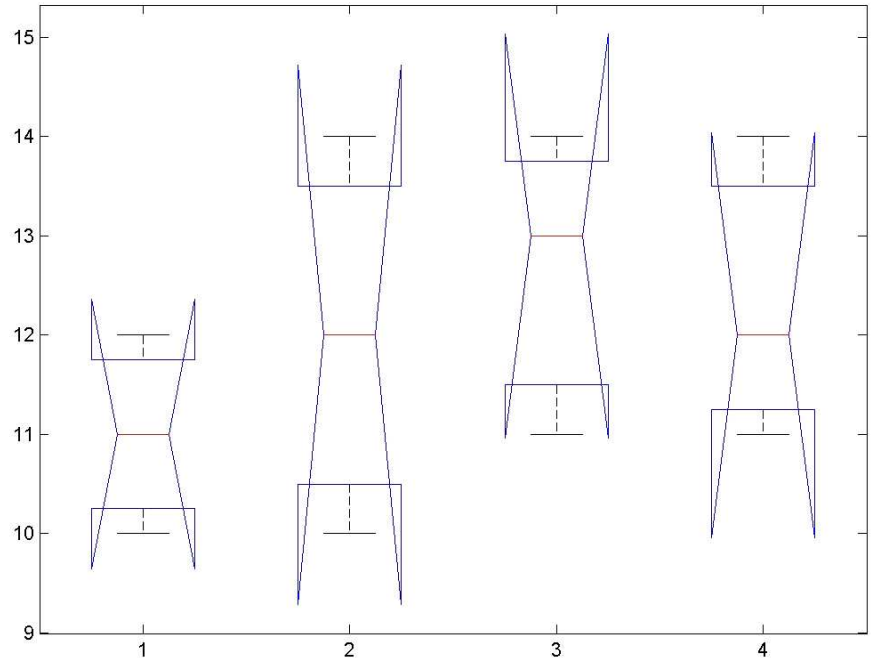
14. LDH assay



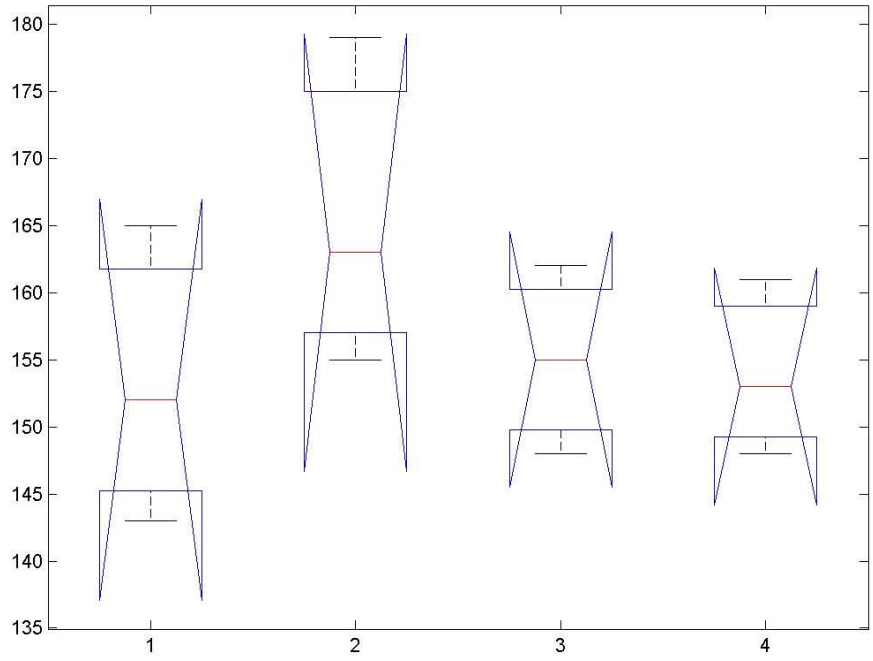
15. Blood pathological analysis
    - ALP



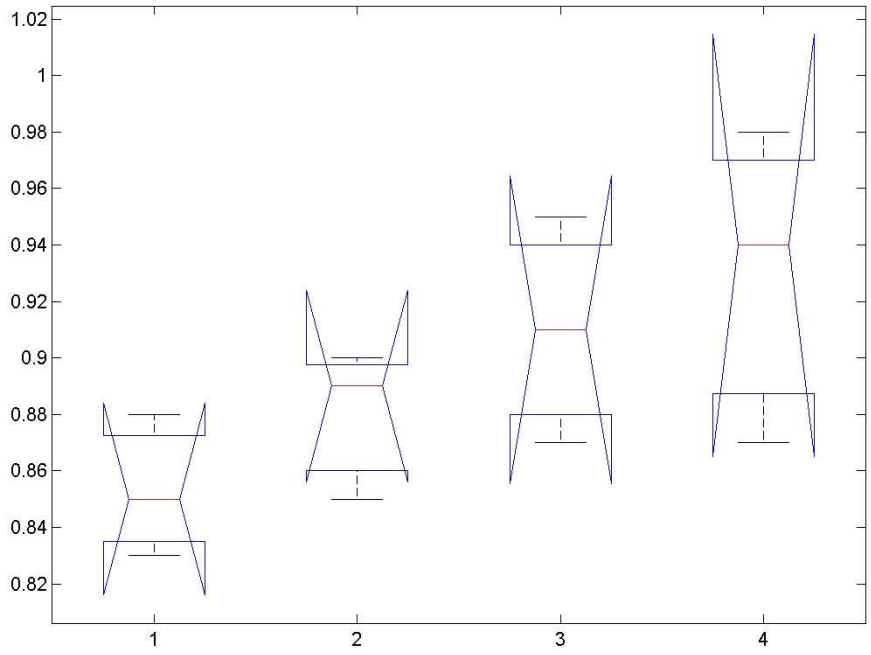
- BUN



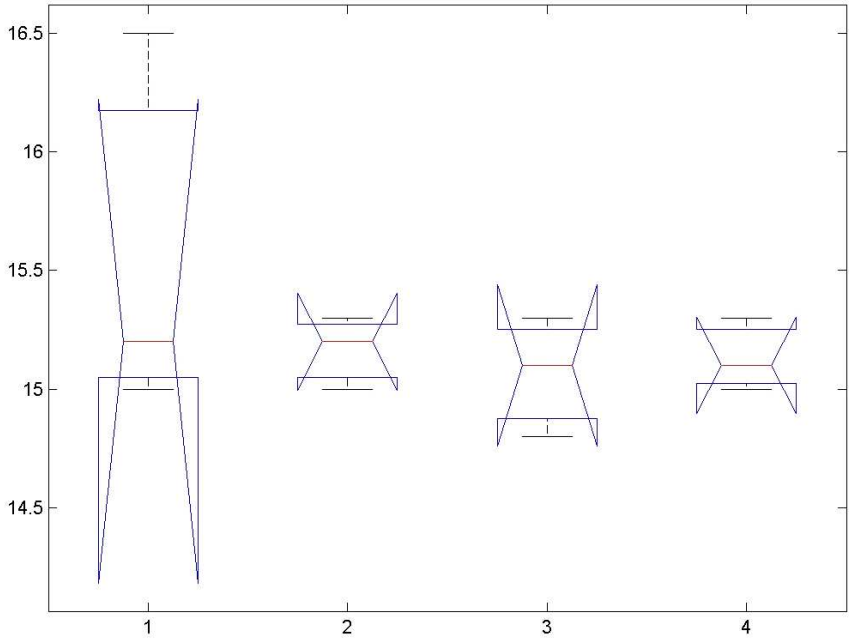
- Cholesterol



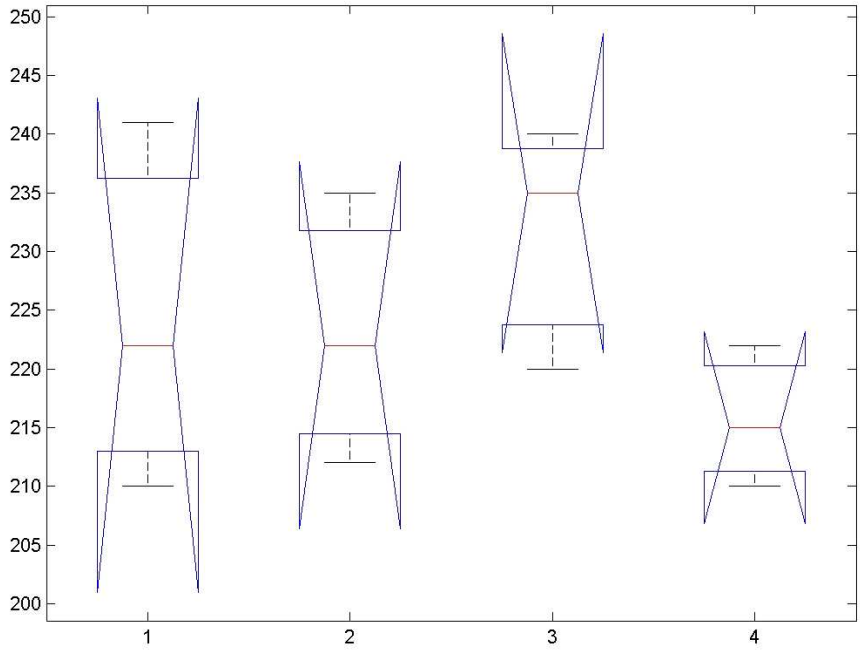
- Creatinine



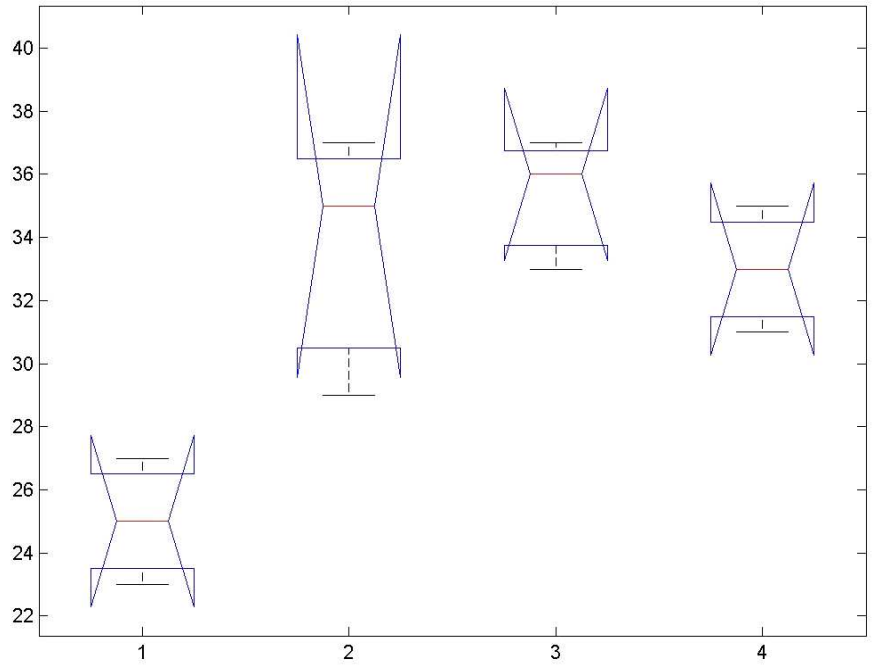
- Haemoglobin



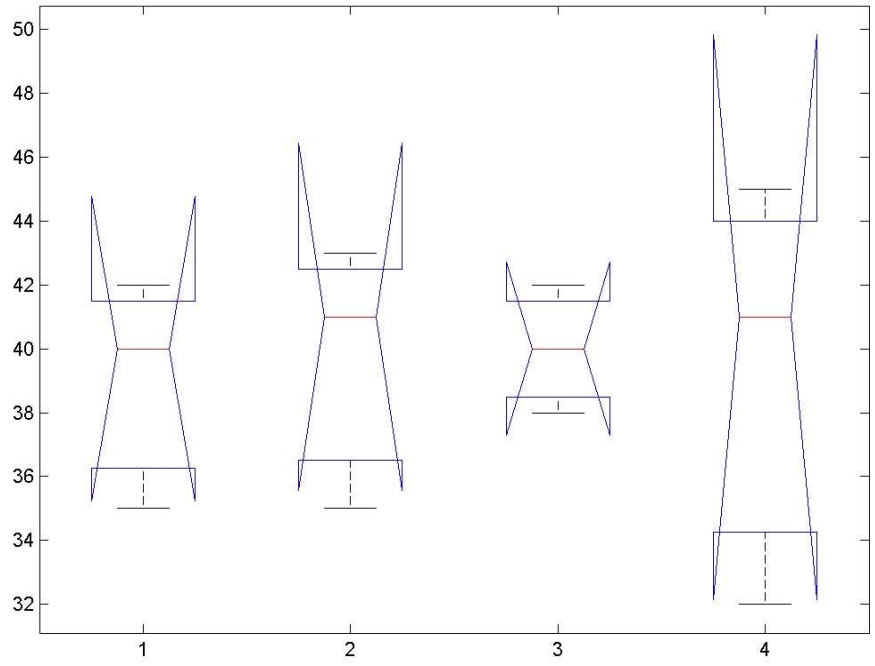
- LDH



- Lymphocyte



- Neutrophils



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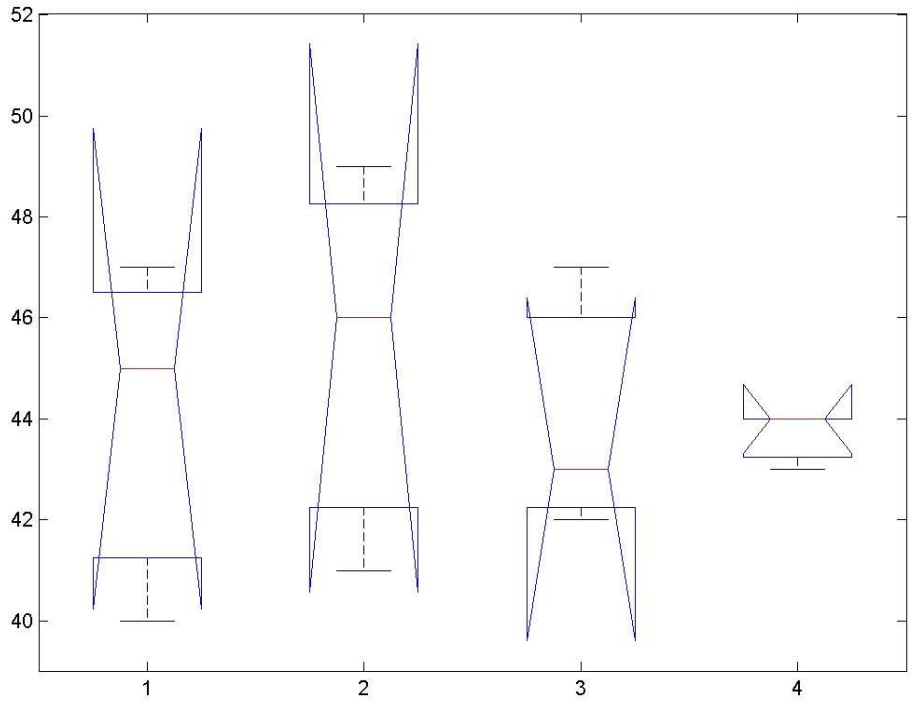
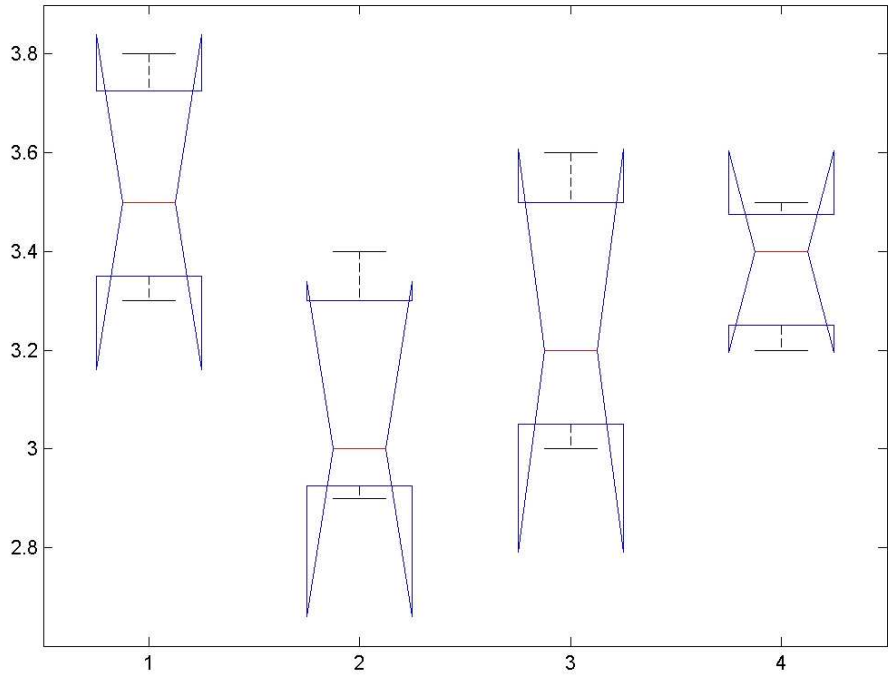
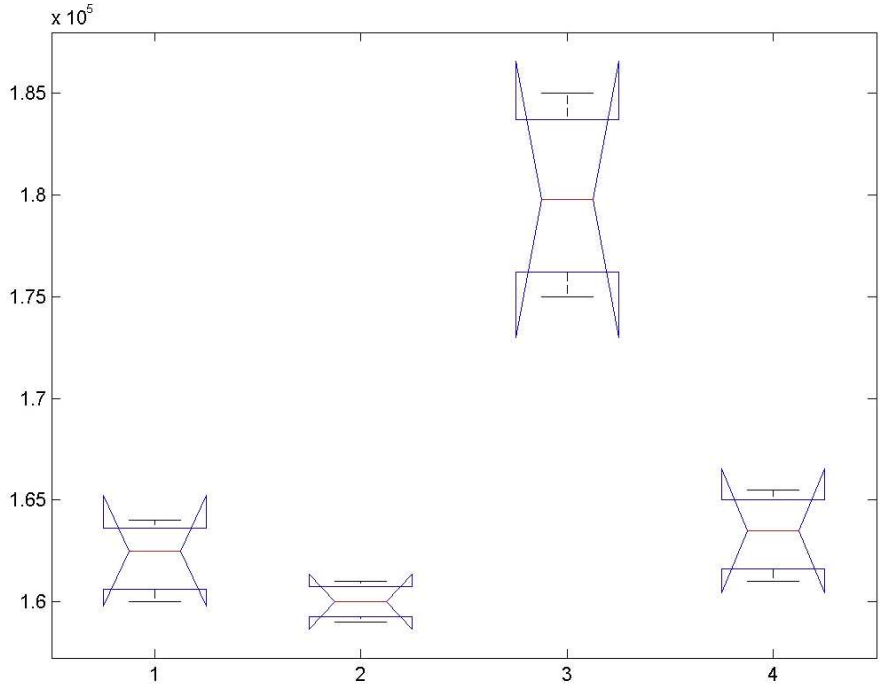


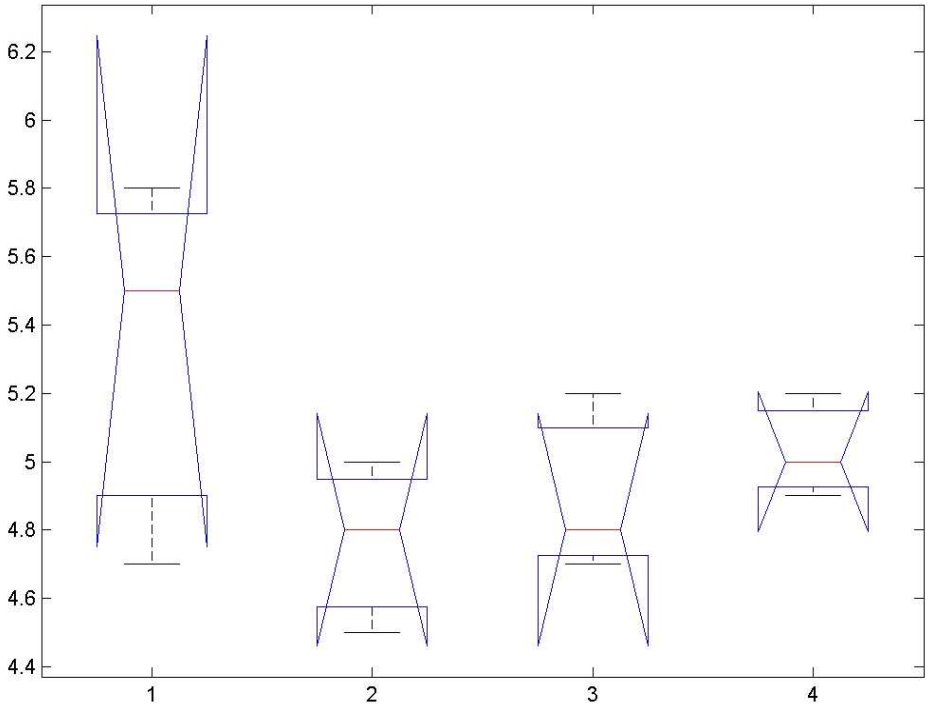
- Phosphorus



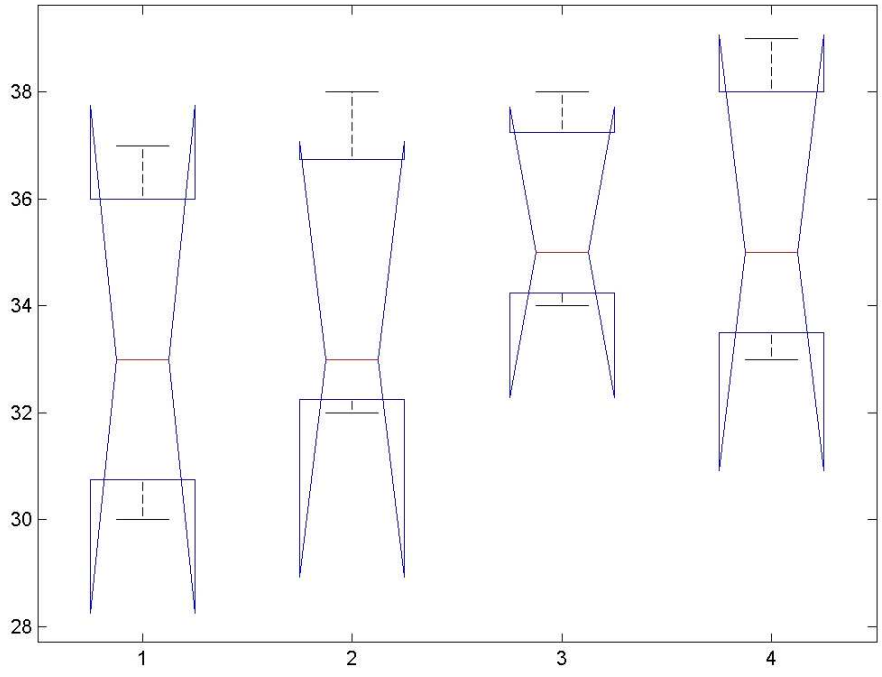
- Platelet



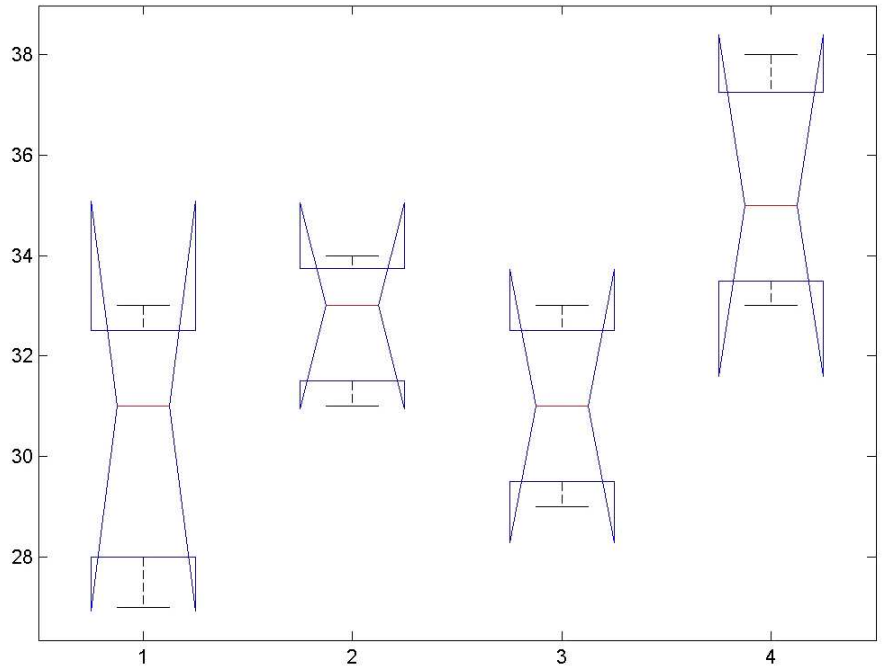
- RBC



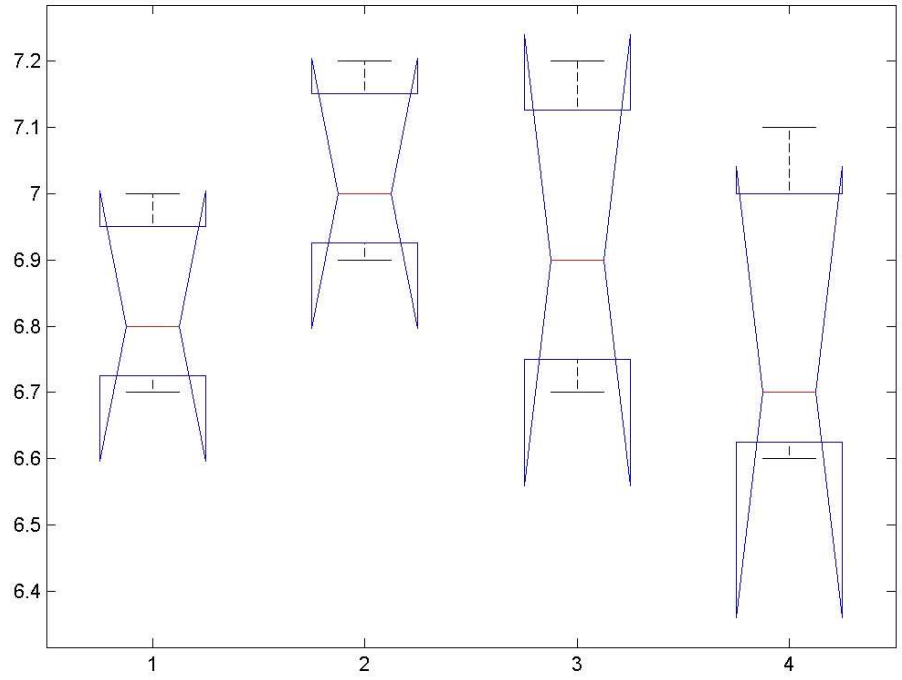
- SGOT



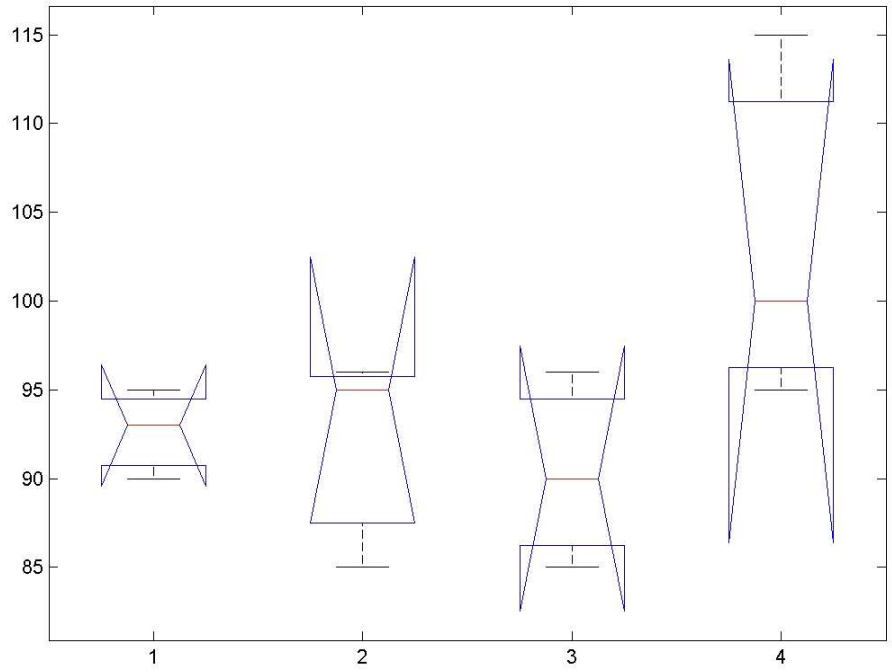
- SGPT



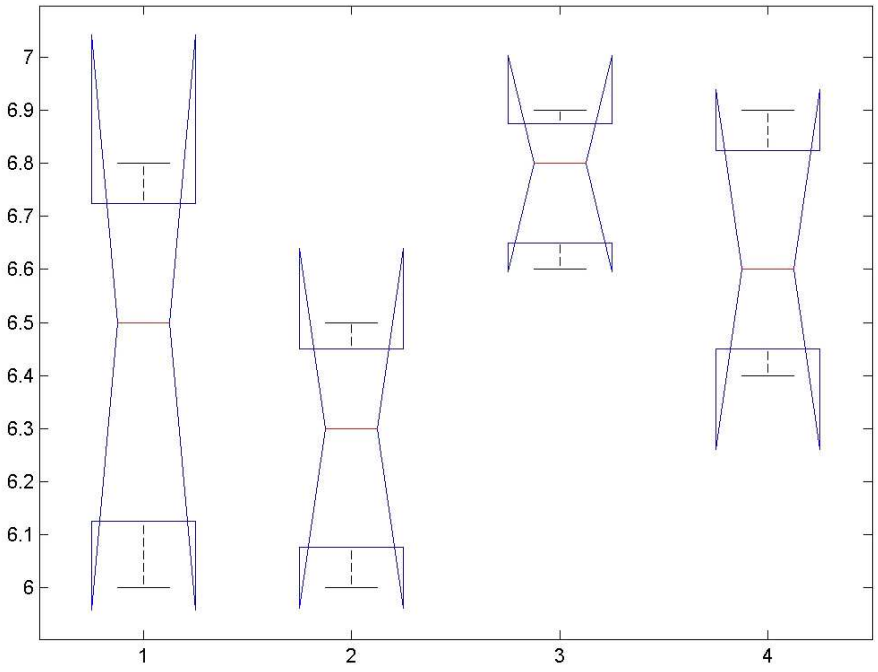
- Total protein



- Triglyceride



- Uric acid



- WBC