

Session 45.

Indicators, hi-tech solutions, and tools to effectively manage cattle

Room: Valdostana – Palazzo Affari 3rd Floor
Chair: Aernouts



Effectiveness of energy dispersive X-ray fluorescence (ED-XRF) for the prediction of mineral elements in individual cow urine

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HEAT STRESS

Physiological condition that occurs when an animal is unable to effectively dissipate excess body heat.



Respiratory rate and body temperature
Incidence of mastitis and ketosis

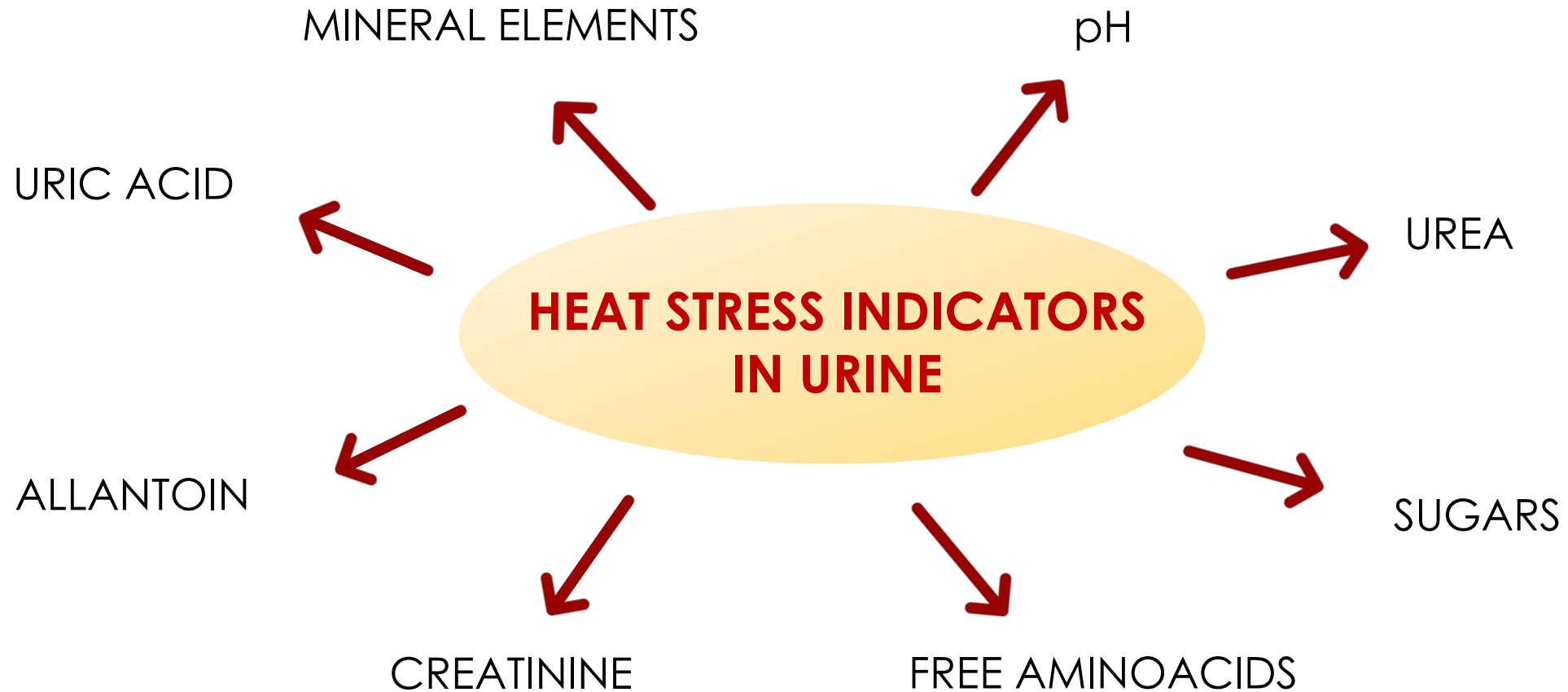


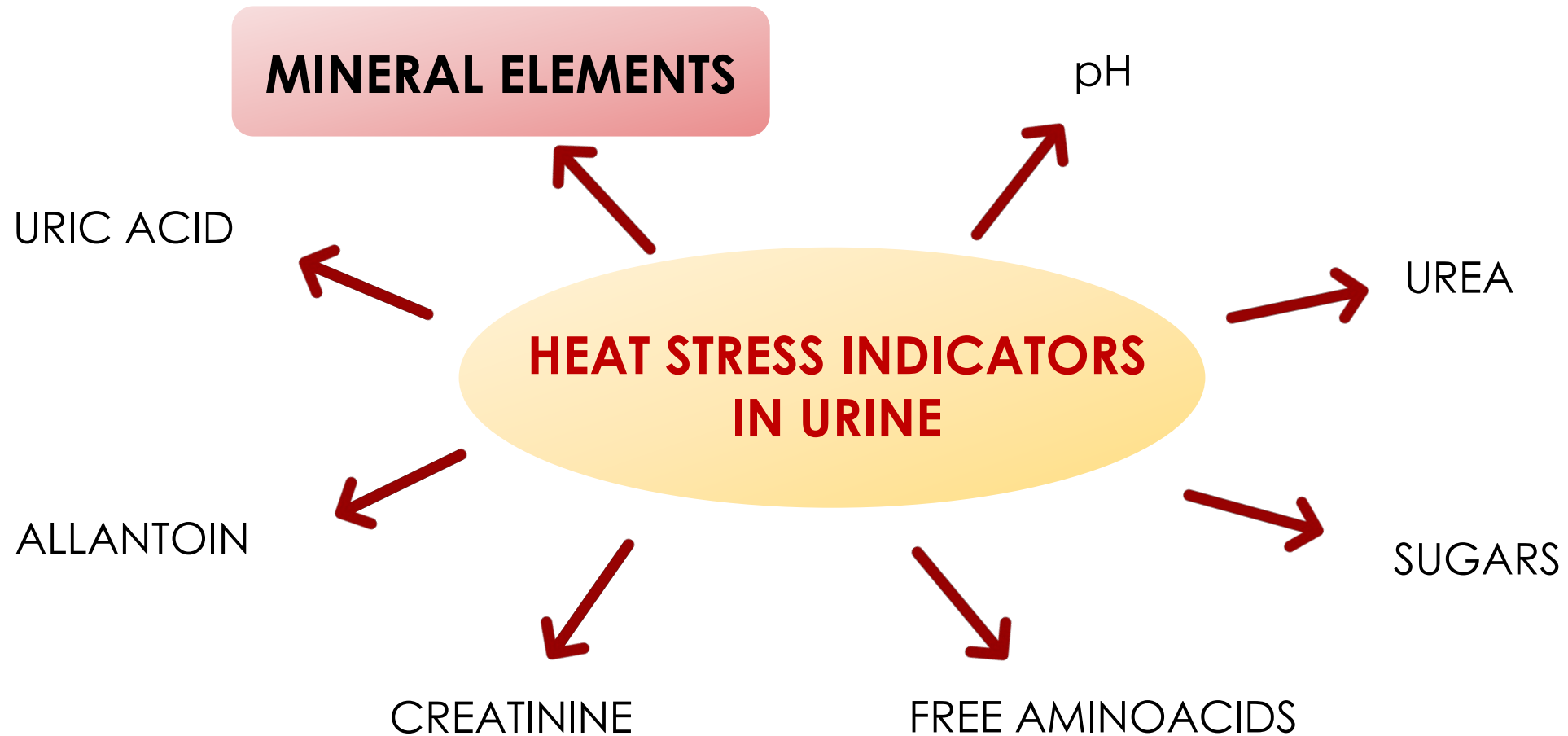
Milk production

Dehydration and electrolyte imbalances
Alteration in milk quality
Reproductive problems

High temperatures
High humidity
Direct sunlight exposure
Lack of ventilation
Physical exertion







REFERENCE METHOD

PREDICTIVE METHOD



REFERENCE METHOD



Inductively coupled plasma optical emission spectrometry (**ICP-OES**)



- ✓ High precision and sensitivity
 - ✓ Expensive equipment
 - ✓ Specialized personnel
 - ✓ Time-consuming

PREDICTIVE METHOD



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PREDICTIVE METHOD



Energy dispersive X-ray fluorescence (**ED-XRF**)
Mid-infrared spectroscopy (**MIRS**)



- ✓ Non-destructive technique
- ✓ Non-specialized personnel
- ✓ Cost-effective
- ✓ Rapid analysis

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Assess the **effectiveness of energy dispersive X-ray fluorescence** for the prediction of:

PERIODIC TABLE OF ELEMENTS

1 H Hydrogen																	2 He Helium				
3 Li Lithium	4 Be Beryllium															5 B Boron	6 C Carbon	7 N Nitrogen	8 O Oxygen	9 F Fluorine	10 Ne Neon
11 Na Sodium	12 Mg Magnesium															13 Al Aluminum	14 Si Silicon	15 P Phosphorus	16 S Sulfur	17 Cl Chlorine	18 Ar Argon
19 K Potassium	20 Ca Calcium	21 Sc Scandium	22 Ti Titanium	23 V Vanadium	24 Cr Chromium	25 Mn Manganese	26 Fe Iron	27 Co Cobalt	28 Ni Nickel	29 Cu Copper	30 Zn Zinc	31 Ga Gallium	32 Ge Germanium	33 As Arsenic	34 Se Selenium	35 Br Bromine	36 Kr Krypton				
37 Rb Rubidium	38 Sr Strontium	39 Y Yttrium	40 Zr Zirconium	41 Nb Niobium	42 Mo Molybdenum	43 Tc Technetium	44 Ru Ruthenium	45 Rh Rhodium	46 Pd Palladium	47 Ag Silver	48 Cd Cadmium	49 In Indium	50 Sn Tin	51 Sb Antimony	52 Te Tellurium	53 I Iodine	54 Xe Xenon				
55 Cs Cesium	56 Ba Barium	57 La Lanthanum	72 Hf Hafnium	73 Ta Tantalum	74 W Tungsten	75 Re Rhenium	76 Os Osmium	77 Ir Iridium	78 Pt Platinum	79 Au Gold	80 Hg Mercury	81 Tl Thallium	82 Pb Lead	83 Bi Bismuth	84 Po Polonium	85 At Astatine	86 Rn Radon				
87 Fr Francium	88 Ra Radium	89 Ac Actinium	104 Rf Rutherfordium	105 Db Dubnium	106 Sg Seaborgium	107 Bh Bohrium	108 Hs Hassium	109 Mt Meitnerium	110 Ds Darmstadtium	111 Rg Roentgenium	112 Cn Copernicium	113 Nh Nihonium	114 Fl Flerovium	115 Mc Moscovium	116 Lv Livermorium	117 Ts Tennessine	118 Og Oganesson				

* 58 Ce Cerium	59 Pr Praseodymium	60 Nd Neodymium	61 Pm Promethium	62 Sm Samarium	63 Eu Europium	64 Gd Gadolinium	65 Tb Terbium	66 Dy Dysprosium	67 Ho Holmium	68 Er Erbium	69 Tm Thulium	70 Yb Ytterbium	71 Lu Lutetium
** 90 Th Thorium	91 Pa Protactinium	92 U Uranium	93 Np Neptunium	94 Pu Plutonium	95 Am Americium	96 Cm Curium	97 Bk Berkelium	98 Cf Californium	99 Es Einsteinium	100 Fm Fermium	101 Md Mendelevium	102 No Nobelium	103 Lr Lawrencium

- SODIUM
- MAGNESIUM
- POTASSIUM
- CALCIUM
- PHOSPHORUS
- SULFUR
- CHLORINE

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Sample collection



9 commercial dairy farms



45 individual urine samples from Holstein Friesian



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Location



Sample collection



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Mineral analysis

ICP-OES



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Mineral analysis

ICP-OES

ED-XRF



ICP-OES reference analysis

1. Mineralization step

Urine samples were added with H_2O_2 and HNO_3 + heated in microwave at 200°C.

2. Dilution and filtration steps

Mineralized samples were added with ultrapure water and filtered into plastic tubes.



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Sample preparation is necessary

3. ICP-OES analysis

Instrument settings, chemical parameters and physical conditions were selected before starting the analysis of mineral elements.



ICP-OES SPECTRO ARCOS (Ametek)

ED-XRF predictive analysis

No preparatory steps are necessary.



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Urine samples were weighed (5 mL)
and placed in **ED-XRF plastic cups**.



ED-XRF predictive analysis

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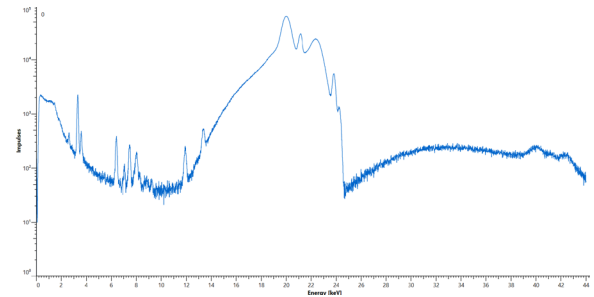
Urine samples were weighed (5 mL) and placed in **ED-XRF plastic cups**.



Information on **type of sample** and **exact weight** was provided to the instrument as the accuracy of the ED-XRF method.



ED-XRF Spectro Xepos 5P (Ametek)



ED-XRF spectrum of an urine sample

Statistical analysis

- ✓ The accuracy of ED-XRF technique was assessed using 2 data sets:

TRAINING SET

70% of the samples

$n = 30$



ICP-OES analysis vs ED-XRF technique



R^2_{TR}

Statistical analysis

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ICP-OES analysis vs mineral concentrations
predicted through the training equations



R^2_{TE} and RPD_{TE}

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ICP-OES analysis vs mineral concentrations
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R^2_{TE} and RPD_{TE}

- ✓ For each mineral, training and testing set were independently prepared.



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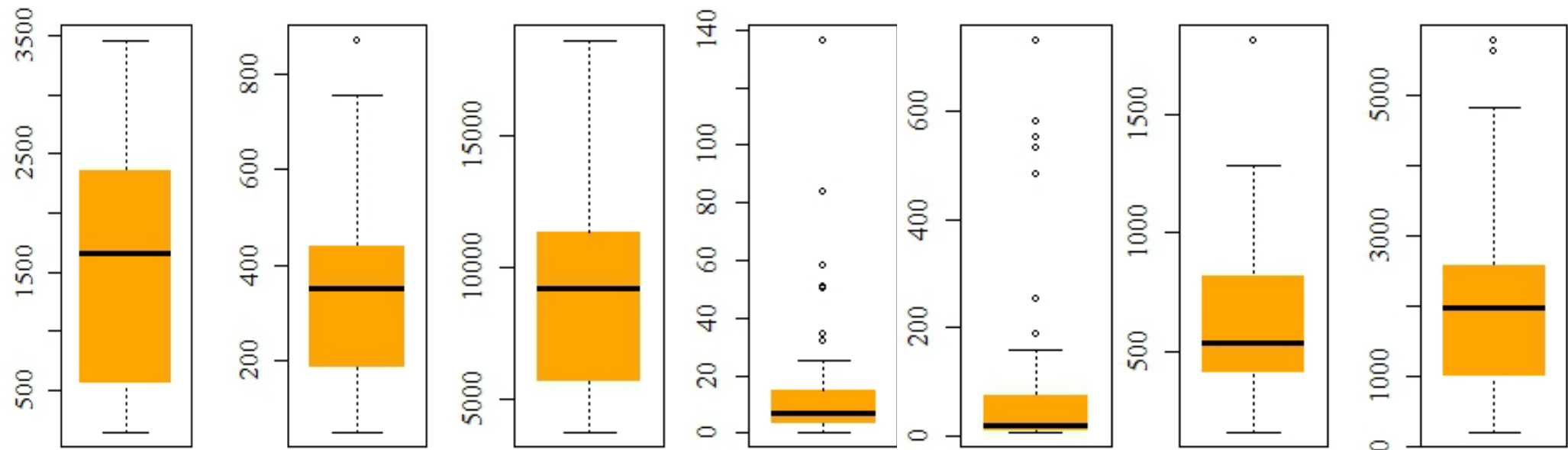
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RESULTS & DISCUSSION

Descriptive statistics of urine mineral elements concentrations measured through ICP-OES

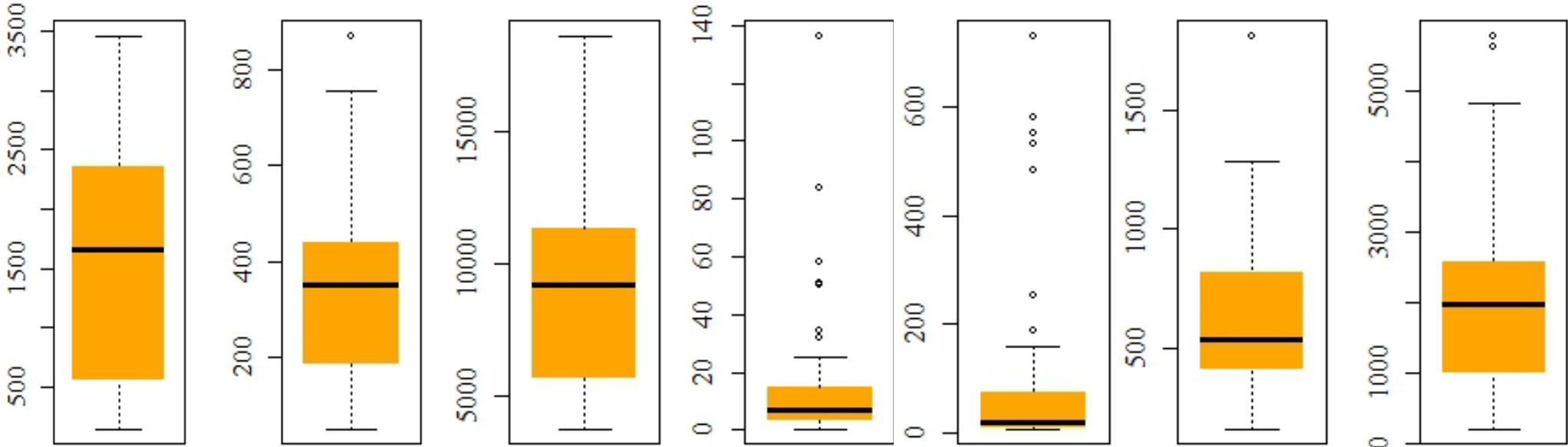
Minerals, mg/kg	Na	Mg	K	Ca	P	S	Cl
Mean (± SD)	1625.96 (± 1003.84)	333.57 (± 198.62)	8961.91 (± 3405.98)	16.00 (± 24.74)	97.14 (± 176.32)	639.60 (± 331.02)	2042.38 (± 1371.29)



RESULTS & DISCUSSION

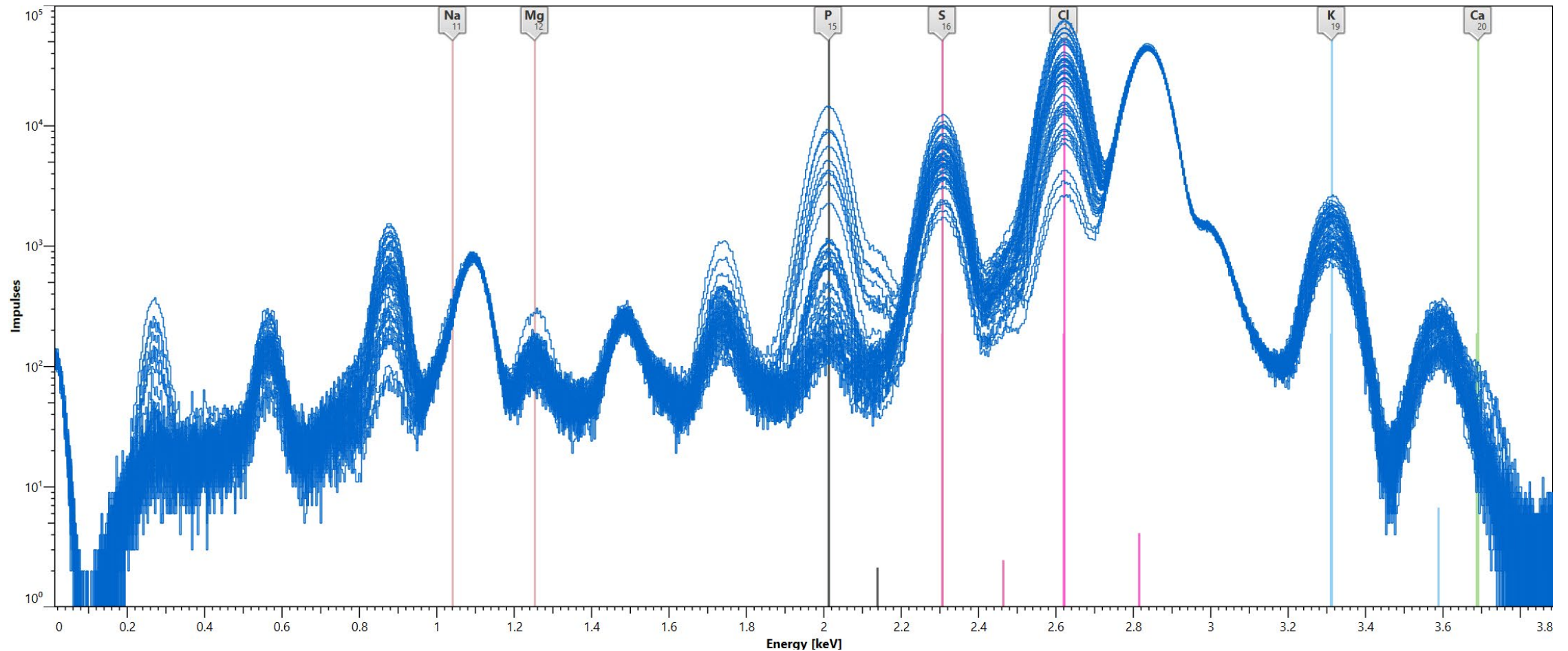
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RESULTS & DISCUSSION

X-ray fluorescence spectrum of urine samples ($n = 45$) and excitation energies associated to each mineral elements



Fitting statistics¹ of the prediction models calculated within training set and testing set

Minerals, mg/kg	Training set		Testing set		
	R ² _{TR}	SE _{TR}	R ² _{TE}	SE _{TE}	RPD _{TE}
Na	0.71	366.87	0.76	495.55	2.10
Mg	0.79	108.48	0.64	172.35	1.10
K	0.83	1,481.42	0.51	2,449.22	1.38
Ca	0.92	8.15	0.63	14.51	1.86
P	0.89	46.04	0.76	144.19	0.96
→ S	0.89	85.99	<u>0.80</u>	161.56	1.69
→ Cl	0.95	269.06	<u>0.89</u>	490.87	2.64

¹R²_{TR}: coefficient of determination in training set; SE_{TR}: standard error in training set; R²_{TE}: coefficient of determination in testing set; SE_{TE}: standard error in testing set; RPD_{TE}: ratio performance to deviation in testing set.



Fitting statistics¹ of the prediction models calculated within training set and testing set

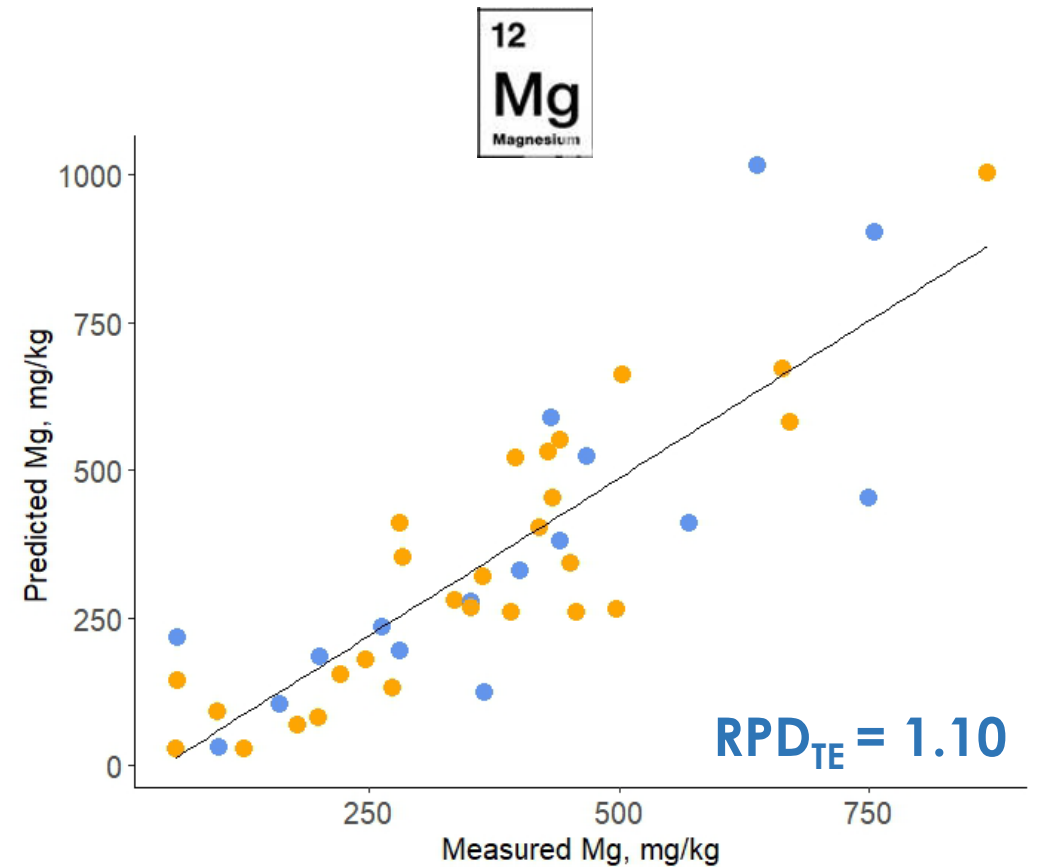
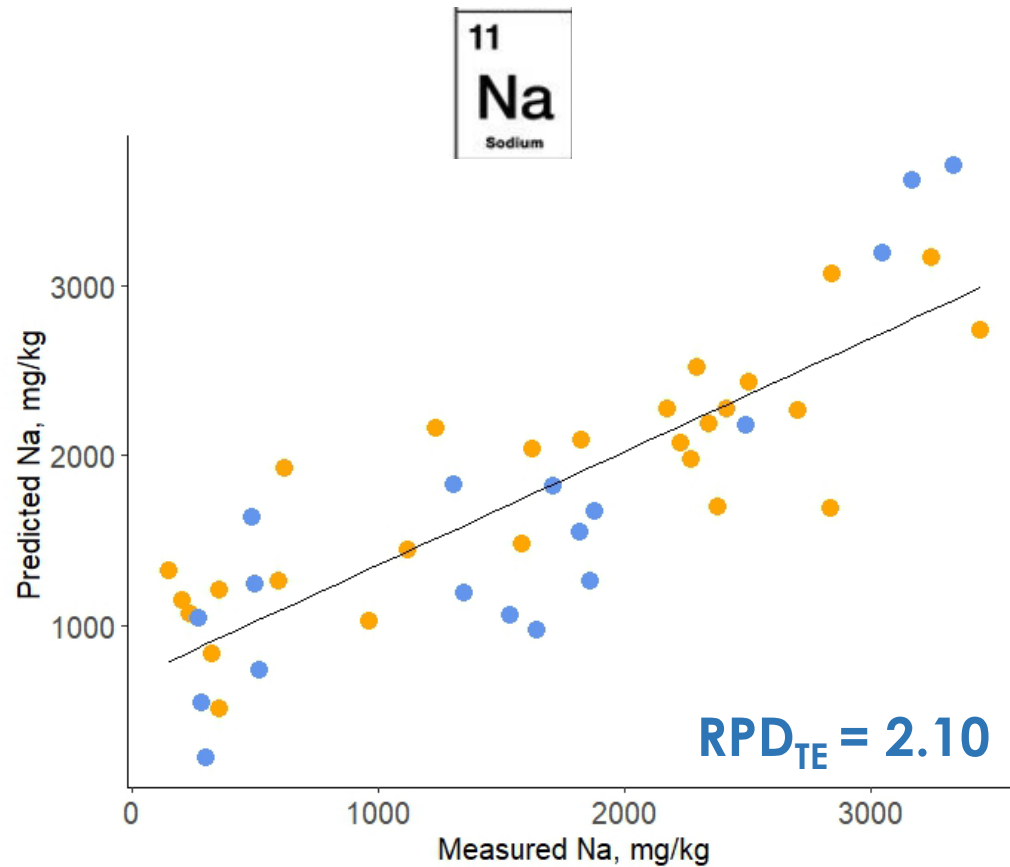
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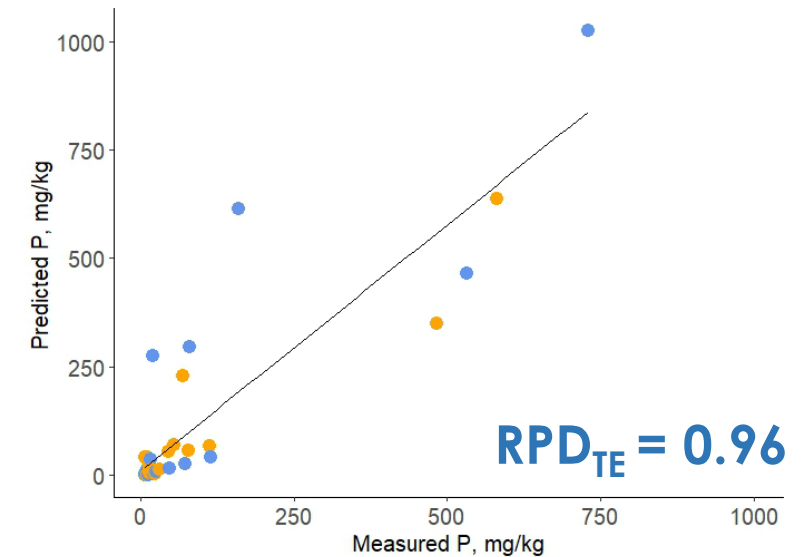
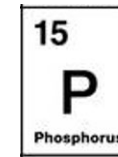
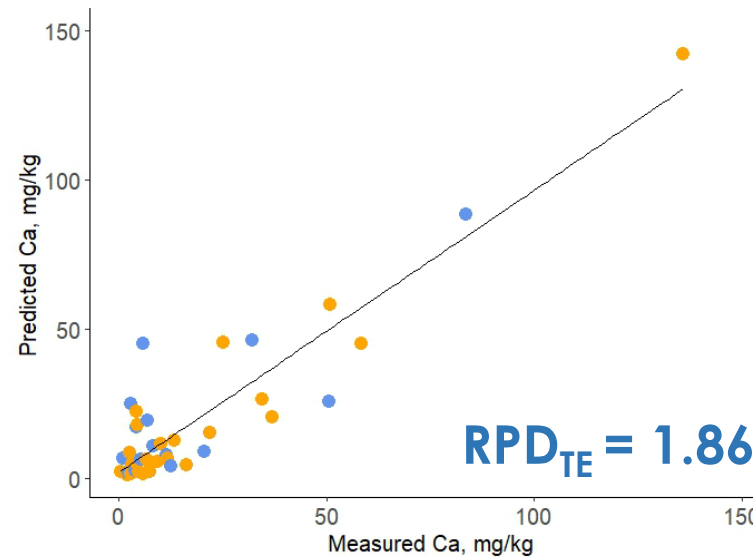
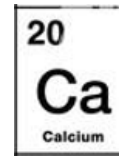
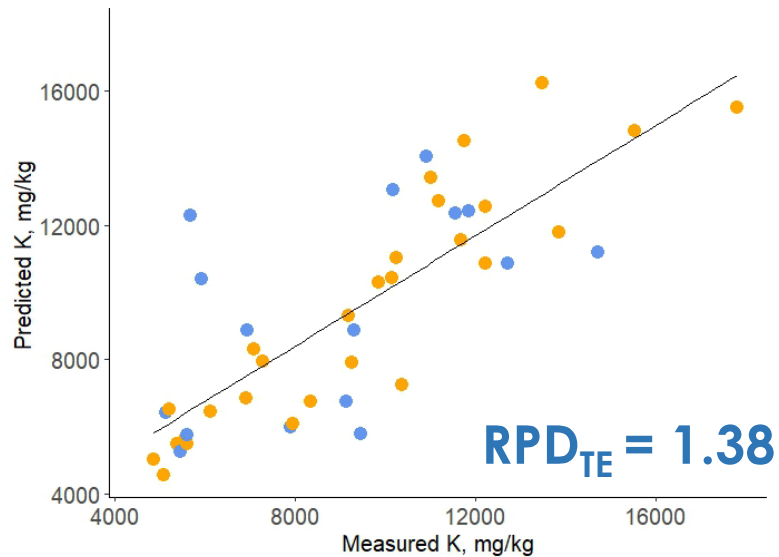
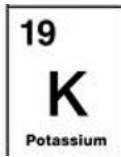


Measured through ICP-OES vs predicted through ED-XRF



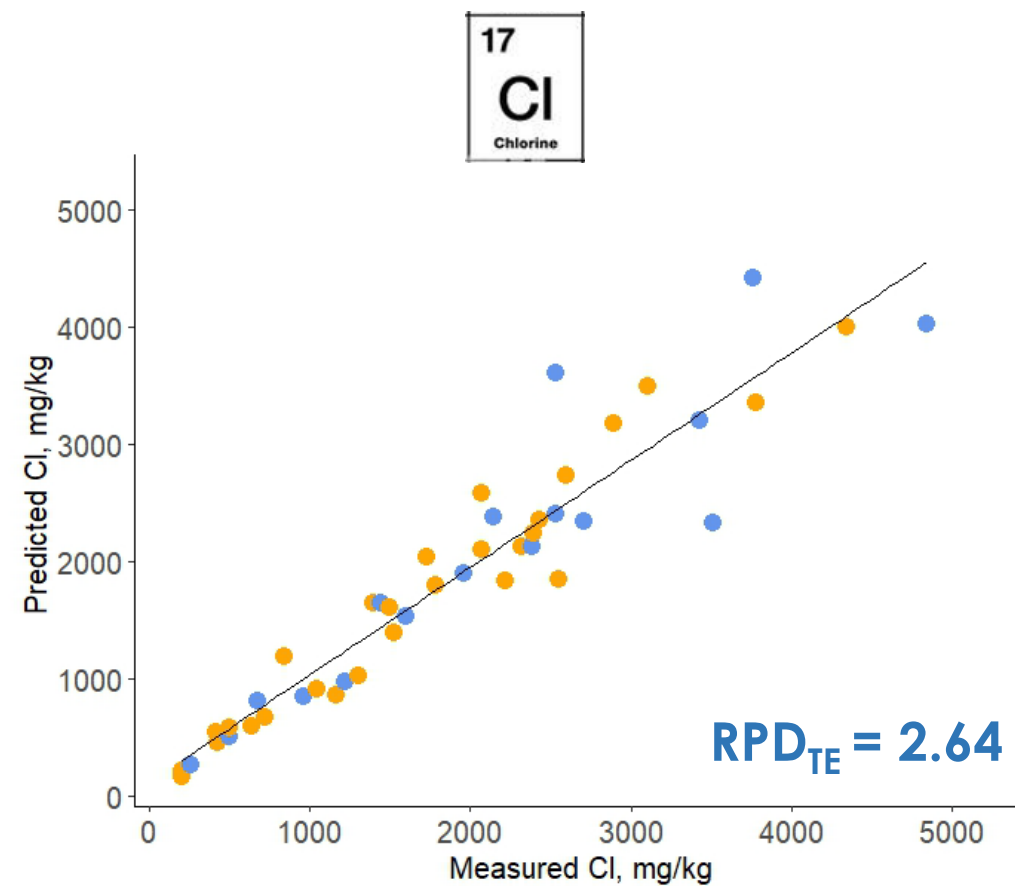
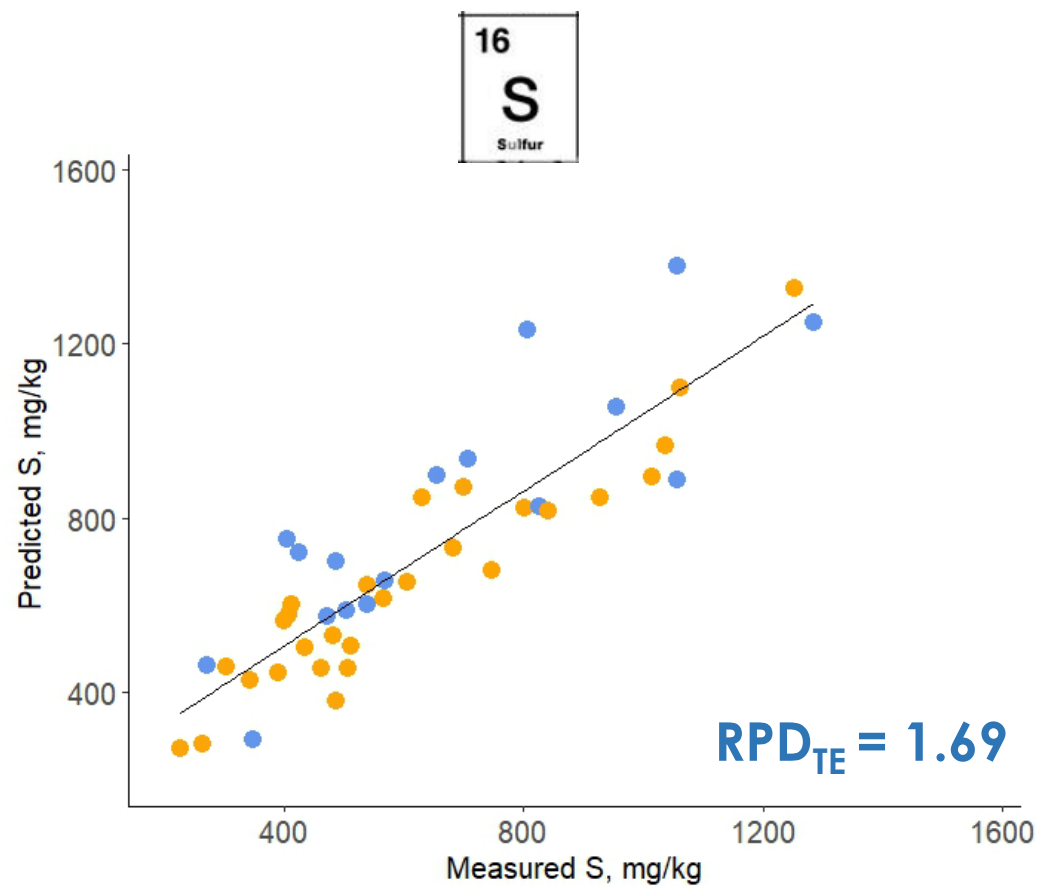


Measured through ICP-OES vs predicted through ED-XRF





Measured through ICP-OES vs predicted through ED-XRF



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CONCLUSIONS

- ✓ Results represent the first step for the development of **rapid technique to predict urine mineral elements** as indicators of heat stress in dairy cows.
- ✓ **Better results** were obtained for **S** and **Cl**.
- ✓ There is **alignment among the training and the testing sets** regarding the good prediction accuracies for **Na, Mg, P, S** and **Cl**.
- ✓ **ED-XRF prediction models** can be used to analyze **unprocessed urine samples** for monitoring heat stress and mineral status in dairy cows.





The 75th EAAP Annual Meeting

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Thank you for your attention!



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DI PADOVA



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