Evaluation of Metal Ion and DNA Recovery from the Surface of Brass Ammunition to Improve STR Profiling

Natalia Czado, M.S.*, Rachel Houston, PhD, Sheree Hughes, PhD

Department of Forensic Science

Sam Houston State University

Huntsville, TX

Sam Houston State University

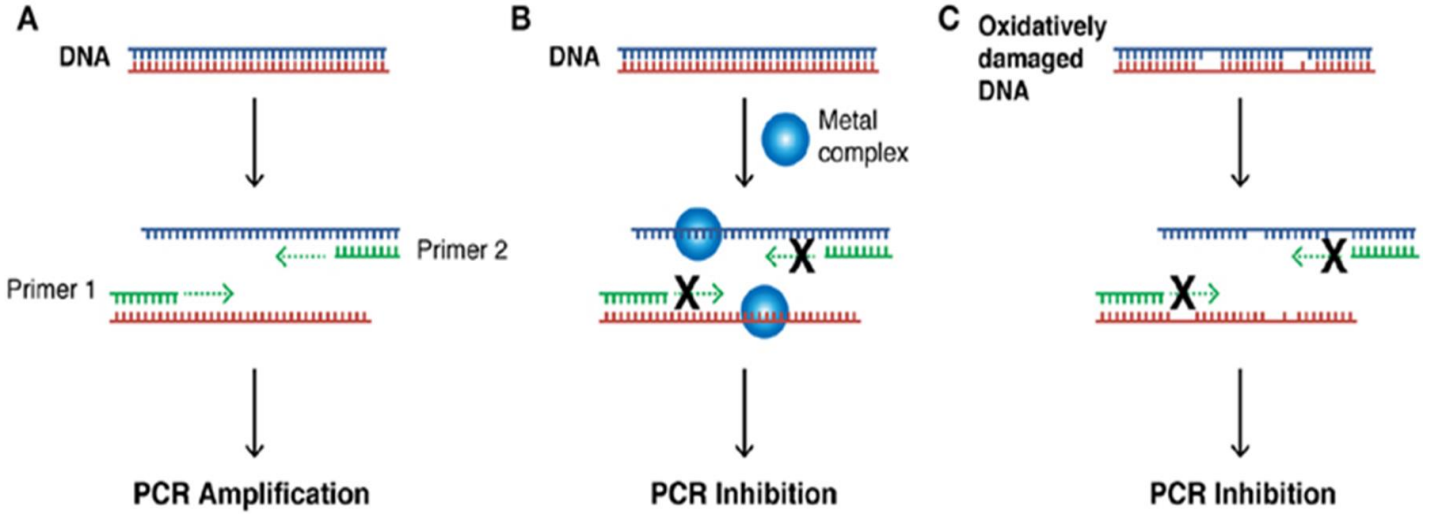
Firearms Evidence

- Firearms and ammunition routinely examined for fingerprints and striations/ impressions
  - Small, uneven deposition surfaces and motion during loading and firing
  - Striations/ impressions mostly links ammunition to weapon

- Trace or “touch” DNA deposited on gun and loaded ammunition
  - Can more definitively identify those who handled weapon and ammunition if STR typing is successful
  - *Difficulties:* low amounts of deposited DNA, possible mixtures, metal ions degrading DNA and inhibiting PCR

Copper Ion Inhibition

- Recovery Issues:
  - Strong Ionic bonds between metals and DNA[1,2]
- PCR Inhibition
  - Metal ion/ DNA complexes[2]
  - Oxidative damage to DNA strands[2]
- Mobility issues during capillary electrophoresis[2]



https://www.researchgate.net/profile/Zara-Molphy/publication/275639140/figure/fig1/AS:267449132122130@1440776250935/A-Illustration-of-steps-involved-in-a-successful-PCR-reaction-denaturation-primer.png

Recovery Methods for Low Template DNA?

- **Double swabbing**[3-5]
  - Increased DNA yield on other types of samples, but inconsistent for ammunition
- **Soaking**[4,6,7]
  - Modified in-house lysis buffer
    - Increased recovery and success, but minimally (~26% successful)
- **Evaluation of extraction methods**
  - Conflicting results for higher yields with organic extraction and silica-based methods[8,9]
  - Carrier RNA increased DNA yields when added to both organic and silica-based methods[10]
- **Examination of chelators**
  - Swabbing with EDTA[11]
  - Rinse/swab with BTMix[3]
- **Direct PCR proposed to overcome DNA loss during processing**
  - Did not overcome metal ion inhibition[12]

Research Aims

- Determine which collection method recovers the most DNA/ STRs

- Identify/ quantify the metal ions co-recovered with DNA and present during different stages of analysis

Materials and Methods- Samples

- 110 rounds of UV sterilized 9 mm brass cartridges
  - Sumbro X-Force 9mm Luger 124 grain Full Metal Jacket
- Spotted with 10 ng of buccal cell DNA (≈ 510 cells/µL)
- Fired by a male law enforcement officer using a 9mm Glock 19

DNA Recovery Methods

1) Water-moistened nylon swabs

2) Water-moistened cotton swabs

3) EDTA-moistened cotton swabs

4) Soaking

5) BTMix rinse/swab

6) microFLOQ® direct swabs

DNA Analysis

PrepFiler Express BTA™ Forensic DNA Extraction Kit	EZ1 DNA Investigator Kit	Quantifiler Trio DNA Quantification Kit
▪ AutoMate Express™ Forensic DNA Extraction System (ThermoFisher Scientific) ▪ Standard elution volume of 50 µL	▪ EZ1 Advanced XL (QIAGEN) ▪ Large Volume Protocol with a final elution volume of 40 µL	▪ 7500 Real-time PCR System (ThermoFisher Scientific)

DNA Analysis

VeriFiler™ Plus PCR Amplification Kit	ABI 3500 Series Genetic Analyzer	GeneMapper® ID-X Software v1.4 (ThermoFisher Scientific)
▪ ProFlex PCR System (ThermoFisher Scientific) ▪ Direct PCR of the microFLOQ swabs was performed in a 10 µL reaction volume	▪ 36 cm capillary array and POP-4 configuration (ThermoFisher Scientific)	▪ Analytical threshold of 100 RFUs

Metal Ion Analysis

- Texas Research Institute for Environmental Studies (TRIES) Facility
  - Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES)

- 8 metal ion panel used on most representative DNA recovery methods:
  1) Copper
  2) Zinc
  3) Lead
  4) Barium
  5) Antimony
  6) Iron
  7) Aluminum
  8) Nickel

Table 1. Metal Ion Concentrations for All Expected Metals Recovered Using Representative Methods

Metal	Fired Casings (mg/kg)	Soaked Fired Casings (mg/kg)	Soaked Unfired Casings (mg/L)	EDTA-Moistened Cotton Double Swab- Fired Casings (mg/kg)	EDTA-Moistened Cotton Double Swab- Unfired Casings (mg/kg)
Copper	411	26.4	19.84	58	17.18
Zinc	198	11.74	5.63	18.54	6.42
Barium	206	3.21	0.087	5.26	1.89
Lead	184	6.38	0.49	7.34	0.22
Iron	120	0.063	0.077	3.00	4.00
Antimony	71.5	2.53	0.025	0.112	0.29
Aluminum	52.9	0.014	0.014	1.25	0.54
Nickel	4.11	0.033	0.004	0.015	0.004

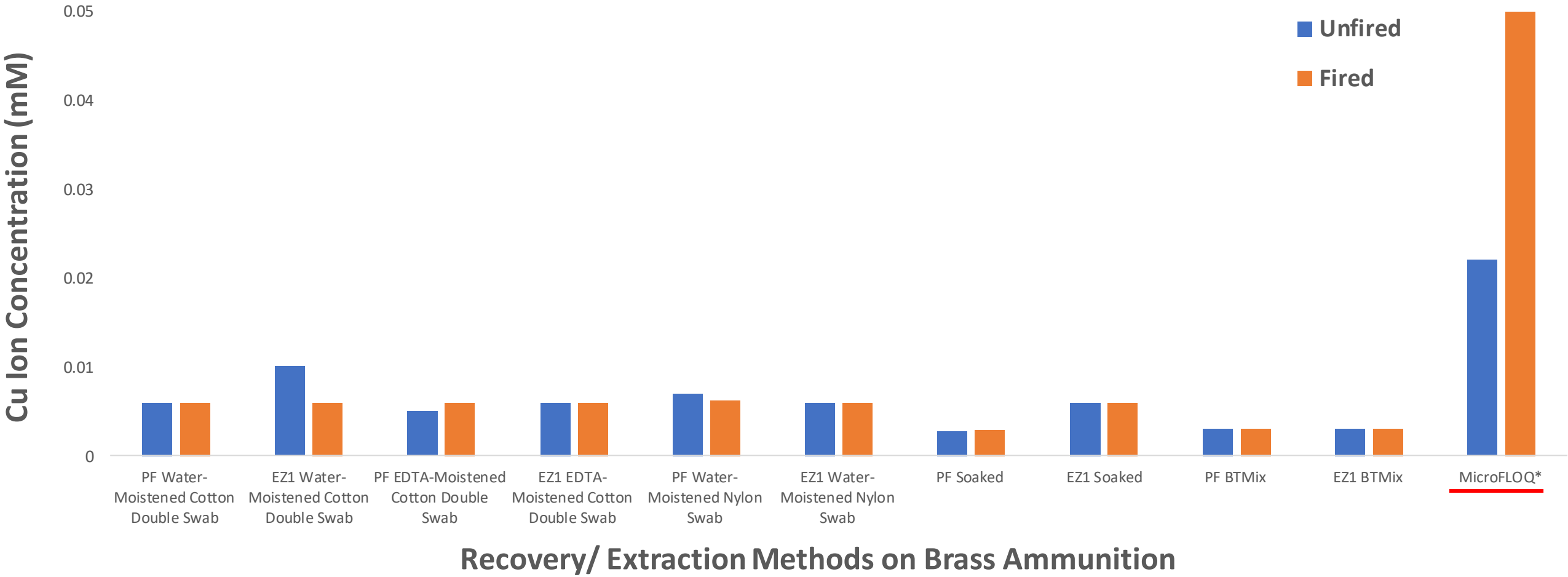
- Copper and zinc were recovered in the highest concentrations
- Only these two metals were analyzed for the remaining collection methods

Table 2. Average concentrations of copper and zinc ions for each recovery method post collection and purification

Recovery Method	Metal Ion Concentrations *Before* Extraction		Metal Ion Concentrations *After* Extraction							
			PrepFiler BTA Express				EZ1 DNA Investigator			
	Avg Cu (mM)	Avg Zn (mM)	Avg Cu (mM)	Cu removed (%)	Avg Zn (mM)	Zn removed (%)	Avg Cu (mM)	Cu removed (%)	Avg Zn (mM)	Zn removed (%)
Water-Moistened Cotton Double Swab- **Unfired**	0.3	0.017	0.006	***98***	0.003	***82.35***	0.01	***96.67***	0.003	***82.35***
Water-Moistened Cotton Double Swab- **Fired**	0.37	0.033	0.006	***98.37***	0.003	***90.91***	0.006	***98.37***	0.003	***90.91***
EDTA-Moistened Cotton Double Swab- **Unfired**	0.27	0.098	0.005	***99.87***	0.003	***96.94***	0.006	***97.78***	0.003	***96.94***
EDTA-Moistened Cotton Double Swab- **Fired**	0.91	0.28	0.006	***99.34***	0.003	***98.93***	0.006	***99.34***	0.003	***98.93***
Water-Moistened Nylon Swab- **Unfired**	0.04	0.005	0.007	***80.56***	0.003	***36.17***	0.006	***83.33***	0.003	***36.17***
Water-Moistened Nylon Swab- **Fired**	0.032	0.015	0.0062	***80.63***	0.003	***80***	0.006	***81.25***	0.003	***80***
Soaked- **Unfired**	0.3	0.09	0.0027	***99.1***	0.0015	***98.26***	0.006	***98***	0.003	***96.51***
Soaked- **Fired**	0.42	0.18	0.0029	***99.31***	0.0015	***99.17***	0.006	***98.57***	0.003	***98.33***
BTMix- **Unfired**	0.06	0.006	0.003	***95***	0.003	***50***	0.003	***95***	0.003	***50***
BTMix- **Fired**	0.07	0.005	0.003	***95.83***	0.003	***66.67***	0.003	***95.83***	0.003	***66.67***
MicroFLOQ- **Unfired**	0.022	0.07	*Direct PCR*							
MicroFLOQ- **Fired**	0.05	0.012								

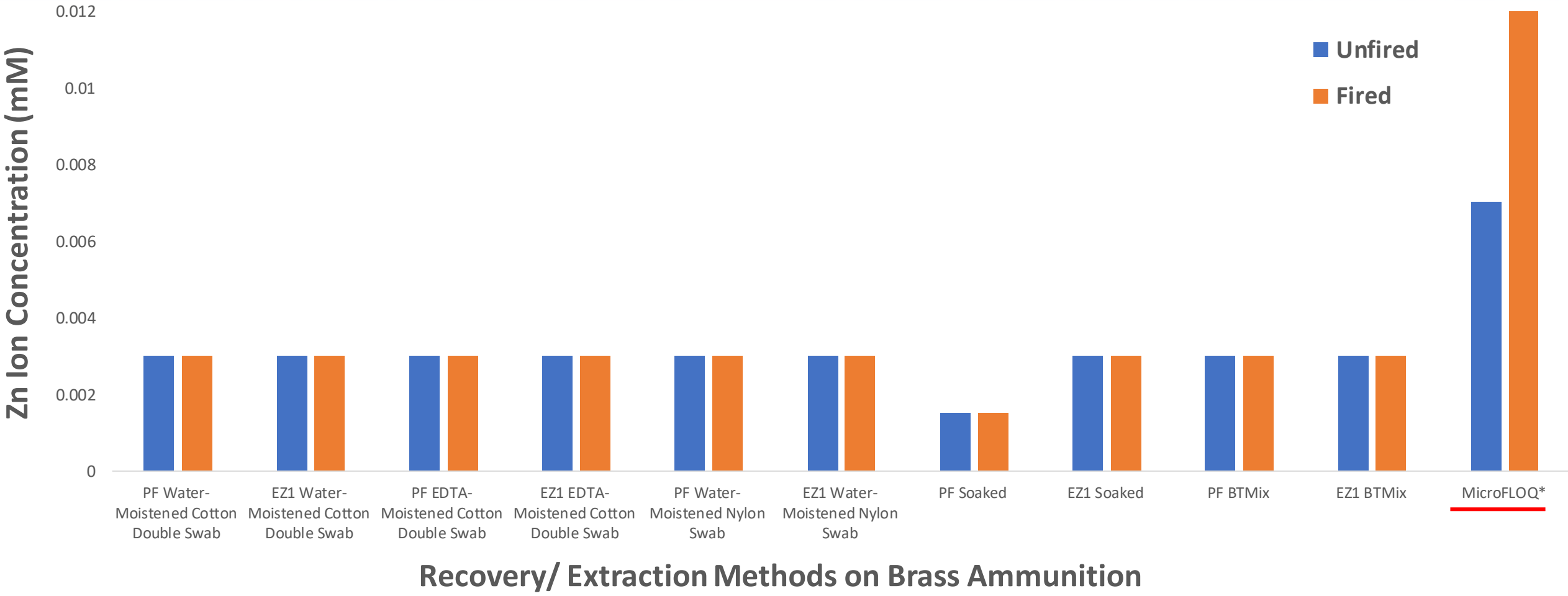
- Highest concentration of metal ions detected after collection and before DNA extraction
- PCR inhibitors present after DNA collection were removed during DNA purification

Fig. 1. Comparison of Copper Concentrations After DNA Purification



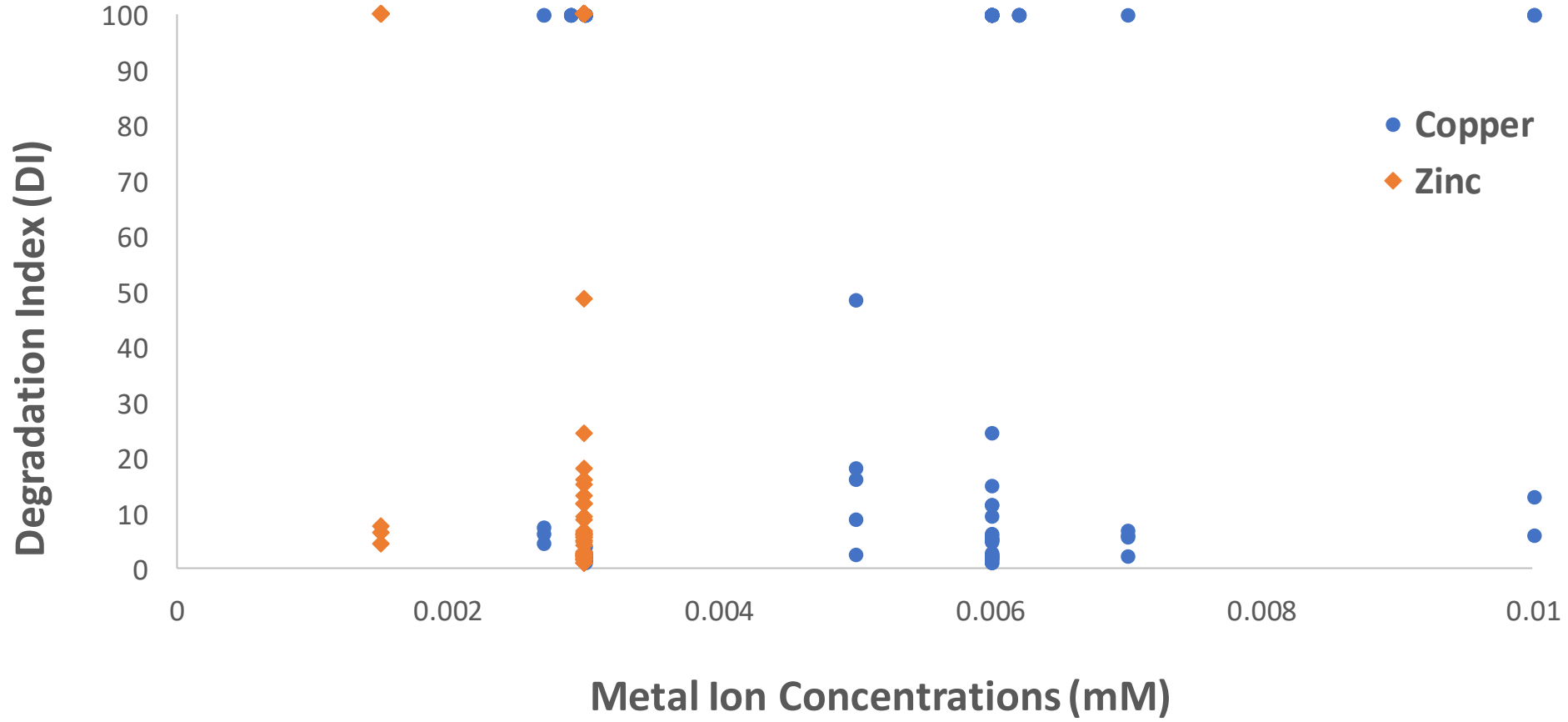
- After DNA purification, similar copper values were obtained for all recovery methods, except microFLOQ swabbed samples
- Direct PCR introduces inhibitors into the amplification reaction

Fig. 2. Comparison of Zinc Concentrations After DNA Purification



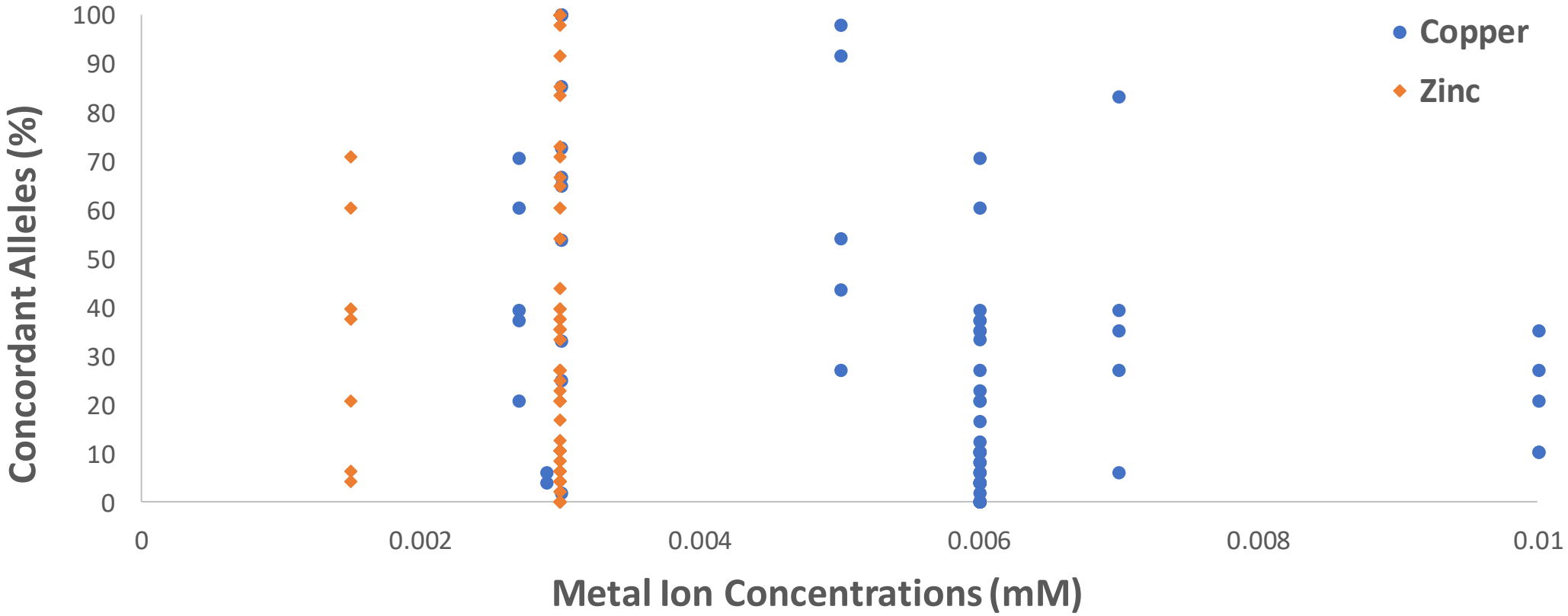
- After DNA purification, similar zinc values were obtained for all recovery methods, except microFLOQ swabbed samples
- Direct PCR introduces inhibitors into the amplification reaction

Fig. 3. Is Ion Concentration and DNA Degradation related?



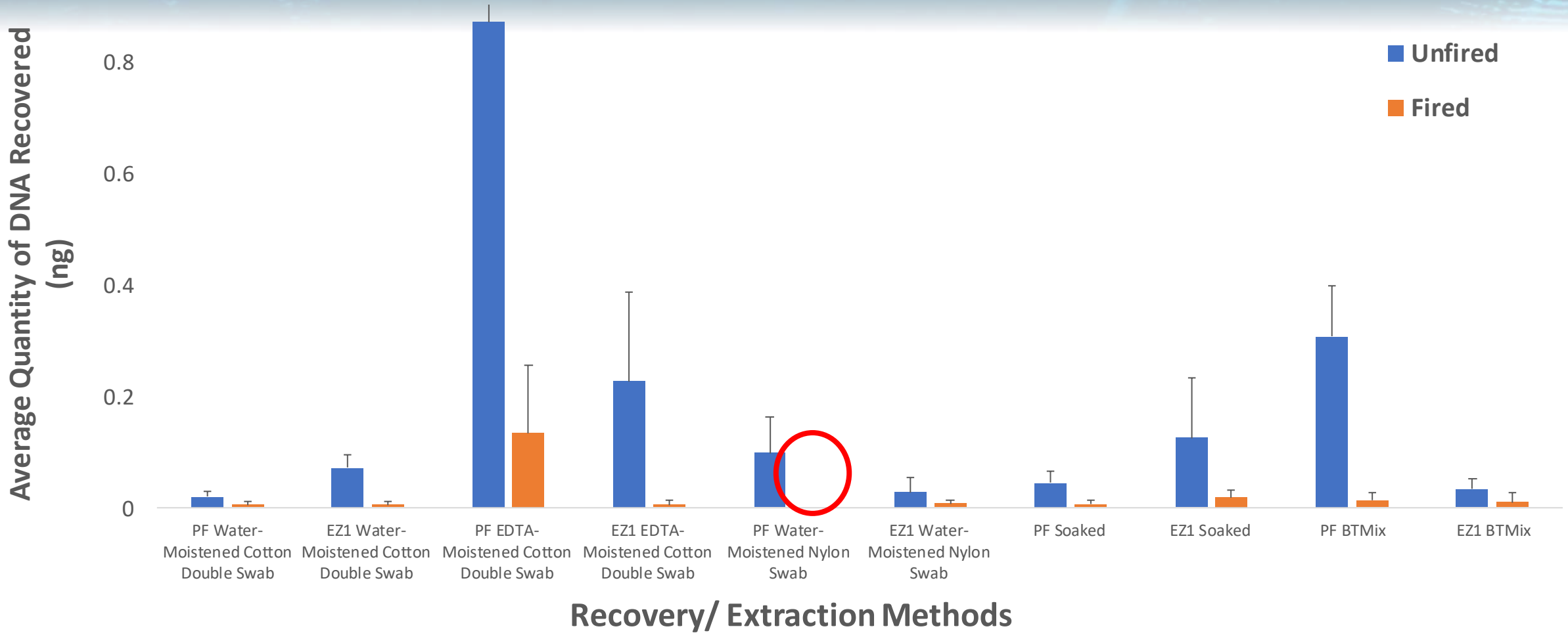
- All samples exhibited some level of degradation
  - Average DI values ranged from 2.5 to 80.4 in samples where both targets were amplified
  - Upper limit of 100 was assigned for samples where the DI was undetermined
- No indication of PCR inhibition during quantification

Fig. 4. Comparison of Ion Concentration to Genotyping Success



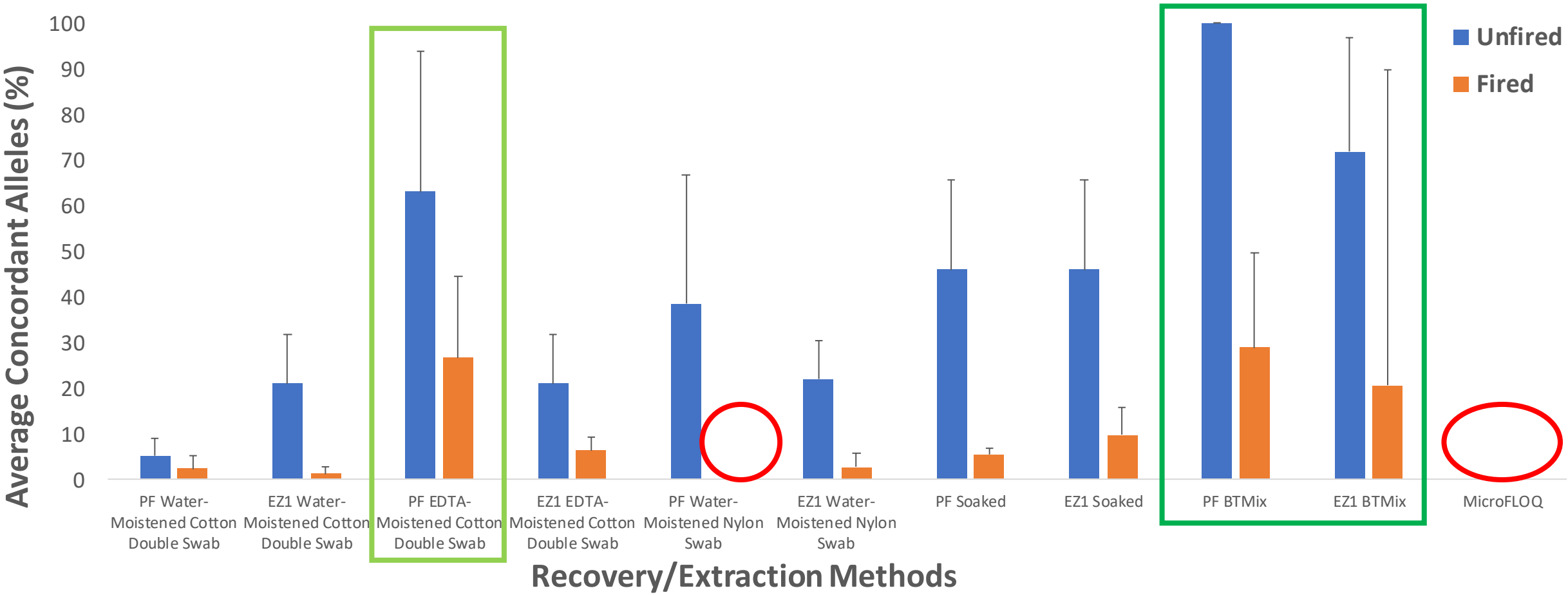
- No relationship was observed between the presence of copper or zinc ions after purification and the percentage of correct alleles genotyped
- Poor genotyping results are most likely the result of damage and degradation of the DNA template due to the initial interaction with the metal ions, rather than their presence after purification

Fig. 5. Comparison of DNA Quantity Recovered with Each Collection Method



- Swabbing with water-moistened nylon swabs was the only method that did not recover amplifiable DNA for fired samples
- The use of chelating agents increased DNA recovery
  - EDTA-moistened cotton swabs
  - BTMix

Fig. 6. Comparison of Profiling Success with Each Collection Method



- microFLOQ swabs were the only recovery method to yield no concordant STR alleles for both fired and unfired samples
- Rinsing/swabbing with BTMix yielded best overall genotyping success

Conclusions

- STR profiles can be obtained from brass ammunition with some success
- Firing increases both metal ion concentrations and DNA damage
  - Decreases genotyping success
- microFLOQ direct swabs not appropriate for recovery of LT-DNA on brass ammunition
  - PCR inhibitors present after DNA collection were removed during DNA purification using other collection methods
- Use of chelating agents (EDTA and BTMix) recommended, followed by soaking
- Examination of mock trace DNA samples
- Further research into the use of next generation sequencing (NGS) is warranted

Acknowledgements

- Ofc. Jonathon Keith- League City Police Department
- Sam Houston State University
  - Department of Forensic Science
  - The Graduate School
- ThermoFisher Scientific

References

1. Bonsu, D.O.M., D. Higgins, and J.J. Austin, *Forensic touch DNA recovery from metal surfaces–A review.* Science & Justice, 2020. **60**(3): p. 206-215.
2. Moreno, L.I. and B.R. McCord, *Understanding metal inhibition: The effect of copper (Cu2+) on DNA containing samples.* Forensic Chemistry, 2017. **4**: p. 89-95.
3. Bille, T.W., Fahrig, G., Weitz, S.M. and Peiffer, G.A.. *An improved process for the collection and DNA analysis of fired cartridge cases*. Forensic Science International: Genetics, 2020. **46:** p.102238.
4. Dieltjes, P., et al., *A sensitive method to extract DNA from biological traces present on ammunition for the purpose of genetic profiling.* International journal of legal medicine, 2011. **125**(4): p. 597-602.
5. Montpetit, S., *Obtaining DNA from ammunition: A review.* Wiley Interdisciplinary Reviews: Forensic Science, 2020. **2**(2): p. e1352.
6. Montpetit, S. and P. O'Donnell, *An optimized procedure for obtaining DNA from fired and unfired ammunition.* Forensic Science International: Genetics, 2015. **17**: p. 70-74.
7. Elwick, Kyleen, et al. *Recovery of DNA from fired and unfired cartridge casings: comparison of two DNA collection methods*. Forensic Science International: Genetics, 2022. **59**: p. 1-11.
8. Prasad, E., et al., *DNA recovery from unfired and fired cartridge cases: A comparison of swabbing, tape lifting, vacuum filtration, and direct PCR.* Forensic science international, 2020. **317**: p. 110507.
9. Horsman-Hall, K.M., et al., *Development of STR profiles from firearms and fired cartridge cases.* Forensic Science International: Genetics, 2009. **3**(4): p. 242-250.
10. Nguyen, C., *Sensitivity Comparison of an Organic Based DNA Extraction Method to a Silica Based DNA Extraction System Utilizing Carrier RNA and Effects of a Post-PCR Purification Process.* 2008.
11. Holland, M.M., et al., *Recovery of mtDNA from unfired metallic ammunition components with an assessment of sequence profile quality and DNA damage through MPS analysis.* Forensic Science International: Genetics, 2019. **39**: p. 86-96.
12. Cavanaugh, S.E. and A.S. Bathrick, *Direct PCR amplification of forensic touch and other challenging DNA samples: a review.* Forensic science International: Genetics, 2018. **32**: p. 40-49.

All in the name of science…

…and dangerous curiosity

-Eliza Victoria

nic003@shsu.edu